## Oral Presentations

<table>
<thead>
<tr>
<th>Time</th>
<th>Author (Classification-Mentor)</th>
<th>Title</th>
<th>Abstract on Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00-8:10</td>
<td>Golly, Elizabeth (VS-Jacob)</td>
<td>HIGHLY DIVERSE METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS (MRSP) ISOLATED FROM CANINES AT THE NCSU VHC</td>
<td>1</td>
</tr>
<tr>
<td>8:12-8:22</td>
<td>Pultorak, Elizabeth (GS-Breitschwerdt)</td>
<td>BARTONELLA SPP. IN CANINE CUTANEOUS HISTIOCYTOMA</td>
<td>2</td>
</tr>
<tr>
<td>8:24-8:34</td>
<td>DiDomenico, Amy (VS-Gookin)</td>
<td>IMPACT OF ENTEROCOCCUS HIRAE ON GROWTH AND INTESTINAL EPITHELIAL CELL ADHESION OF ENTEROPATHOGENIC E. COLI</td>
<td>3</td>
</tr>
<tr>
<td>8:36-8:46</td>
<td>Lennon, Elizabeth (GS-Moeser)</td>
<td>MAST CELLS PLAY A PROTECTIVE ROLE IN SPONTANEOUS COLITIS</td>
<td>4</td>
</tr>
<tr>
<td>8:48-8:58</td>
<td>Mackey, Emily (GS-Moeser)</td>
<td>SEX-SPECIFIC DEGRANULATION RESPONSES OF MAST CELLS</td>
<td>5</td>
</tr>
<tr>
<td>9:00-9:10</td>
<td>Sills, Shane (VS-Sannes)</td>
<td>EXPRESSION OF WNT-5A SIGNALING PROTEIN IN IDIOPATHIC PULMONARY FIBROSIS</td>
<td>6</td>
</tr>
<tr>
<td>9:12-9:22</td>
<td>Tokarz, Debra (GS-Yoder)</td>
<td>ZEBRAFISH LARVAE REVEAL A NOVEL MEDIATOR OF MACROPHAGE CHEMOTAXIS</td>
<td>7</td>
</tr>
<tr>
<td>9:24-9:34</td>
<td>Dirscherl, Hayley (GS-Yoder)</td>
<td>CHARACTERIZATION OF THE Z LINEAGE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENES IN ZEBRAFISH</td>
<td>8</td>
</tr>
<tr>
<td>9:36-9:46</td>
<td>Stranahan, Lauren (VS-Blikslager)</td>
<td>REDUCTION IN THE NUMBER OF PROGENITOR AND STEM CELLS IN CASES OF LARGE COLON VOLVULUS</td>
<td>9</td>
</tr>
<tr>
<td>9:48-9:58</td>
<td>Boss, Mary-Keara (GS-Dewhirst)</td>
<td>TUMOR MICROENVIRONMENTAL RESPONSES TO RADIATION MARGINAL MISS</td>
<td>10</td>
</tr>
<tr>
<td>1:00-1:10</td>
<td>Frantz, Eva (VS-Sherman)</td>
<td>THE USE OF TRAZODONE TO REDUCE FELINE TRAVEL ANXIETY AND INCREASE VETERINARY EXAM TRACTABILITY</td>
<td>11</td>
</tr>
<tr>
<td>1:12-1:22</td>
<td>Kasten, Jennifer (HO-Campbell)</td>
<td>HEMODYNAMICS AND SEDATIVE EFFECTS OF ORAL TRANSMUCOSAL DETOMIDINE GEL IN DOGS AND ITS REVERSAL WITH ATIPAMEZOLE</td>
<td>12</td>
</tr>
<tr>
<td>1:24-1:34</td>
<td>McKelvey, Katherine (HO-Bailey)</td>
<td>A COMPARATIVE EFFICACY TRIAL FOR TRIMETHOPRIM-SULFAMETHOXAZOLE, GENTAMICIN, AND PENICILLIN USING AN EX VIVO MODEL OF GESTATIONAL DISEASE</td>
<td>13</td>
</tr>
<tr>
<td>1:36-1:46</td>
<td>Warren, Chelsea (VS-Foster)</td>
<td>USE OF A NOVEL COLLECTION METHOD TO MEASURE ACTIVE DRUG CONCENTRATIONS IN THE GI TRACT OF CATTLE TO ASSESS RISK OF ANTIMICROBIAL RESISTANCE IN ENTERIC BACTERIA</td>
<td>14</td>
</tr>
<tr>
<td>1:48-1:58</td>
<td>Charlton, Anna (VS- Matthews)</td>
<td>THE CANINE SACROILIAC JOINT ANGLE: BREED VARIATIONS AND RELATIONSHIP TO LUMBOSACRAL, PELVIC AND STIFLE ANGLES</td>
<td>15</td>
</tr>
<tr>
<td>2:00-2:10</td>
<td>Mzyk, Philip (GS-McGahan)</td>
<td>HYPOXIA AND RETROMER AS POTENTIAL REGULATORS OF POLARIZED AMYLOID PRECURSOR PROTEIN EXPRESSION IN RPE CELLS</td>
<td>16</td>
</tr>
<tr>
<td>Time</td>
<td>Author (Classification-Mentor)</td>
<td>Title</td>
<td>Abstract on Page</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>2:12-2:22</td>
<td>Tomas, Andrea (HO-Lascelles)</td>
<td>STIFLE ARTHROTOMY: A NEW MODEL OF SURGICAL ORTHOPEDIC PAIN IN THE DOG</td>
<td>17</td>
</tr>
<tr>
<td>2:24-2:34</td>
<td>Ward, Jessica (HO-Keene)</td>
<td>COMPLICATION RATES ASSOCIATED WITH PERMANENT TRANSVENOUS PACEMAKER IMPLANTATION IN DOGS WITH HIGH-GRADE ATRIIOVENTRICULAR BLOCK PERFORMED DURING NORMAL BUSINESS HOURS VERSUS AFTER HOURS (97 CASES, 2002-2012)</td>
<td>18</td>
</tr>
<tr>
<td>2:36-2:46</td>
<td>Webb, Kyle (HO-Neel)</td>
<td>CYTOCHEMICAL CHARACTERIZATION OF BLOOD LEUKOCYTES IN ELASMOBRANCHS</td>
<td>19</td>
</tr>
<tr>
<td>2:48-2:58</td>
<td>Jiamachello, Katrina (VS-Lascelles)</td>
<td>QUESTIONNAIRE BASED EVALUATION OF FACTORS INFLUENCING OWNER PARTICIPATION IN CLINICAL RESEARCH WITH CATS</td>
<td>20</td>
</tr>
</tbody>
</table>

VS = Veterinary Student, HO = House Officer, GS = Graduate Student

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### Poster Assignments

**Graduate 10:00-11:00**

<table>
<thead>
<tr>
<th>Poster #</th>
<th>Author (Classification-Mentor)</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Borgmann, Matthew (VS-Remillard, Saker)</td>
<td>CHARACTERIZING THE RELATIONSHIP BETWEEN ADIPOSITY-RELATED PROTEINS AND BODY DEPOSITION OF FAT MASS IN DOGS</td>
</tr>
<tr>
<td>2</td>
<td>Boykin, Kimberly (VS-Orndorff)</td>
<td>LISTERIA MONOCYTOGENES WALL TEICHOIC ACID COMPOSITION AND CELL-TO-CELL SPREAD</td>
</tr>
<tr>
<td>3</td>
<td>Braxton, Alicia (VS - Hauck)</td>
<td>SEQUENCING FOUR MISMATCH REPAIR GENES IN BOXERS TO IDENTIFY MUTATIONS CONTRIBUTING TO A CANCER SUSCEPTIBILITY SYNDROME</td>
</tr>
<tr>
<td>4</td>
<td>D'Costa, Susan (NS - Moeser)</td>
<td>CORTICOTROPIN RELEASING FACTOR 2 REGULATES IGE-MEDIATED MAST CELL DEGRANULATION, ANAPHYLAXIS AND INTESTINAL PERMEABILITY</td>
</tr>
<tr>
<td>5</td>
<td>Dunnigan, Sarah (VS-Borst)</td>
<td>A CHICK EMBRYO LETHALITY TEST FOR ENTEROCCUS CECORUM VIRULENCE</td>
</tr>
<tr>
<td>6</td>
<td>Ferrick, Gregory (VS-Law)</td>
<td>THE RIPPLING EFFECTS OF ECOTOXICOLOGICAL ENDOCRINE DISRUPTING COMPOUNDS ON FISH IN NORTH CAROLINA WATER SYSTEMS</td>
</tr>
<tr>
<td>7</td>
<td>Francher, Taylor (VS-Dorman)</td>
<td>MANGANESE OLFATORY TOXICITY: PHASE I DEVELOPMENT OF A RAT TWO ODOR GO/NO-GO OLFATORY DISCRIMINATION TASK</td>
</tr>
<tr>
<td>8</td>
<td>Gruen, Margaret (GS-Lascelles)</td>
<td>CLINICAL TRIAL APPLICATION OF A CLINICAL PHENOMENON: DETERIORATION FOLLOWING WITHDRAWAL OF ACTIVE MEDICATION FOR THE TREATMENT OF CHRONIC PAIN IN CATS WITH DEGENERATIVE JOINT DISEASE</td>
</tr>
<tr>
<td>Poster #</td>
<td>Author (Classification-Mentor)</td>
<td>Title</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>9</td>
<td>Heinz, Jessica (VS-Bailey)</td>
<td>RESPONSE OF EQUINE CHORIOALLANTOIS TO INFLAMMATORY STIMULI AND ANTI-INFLAMMATORY DRUGS</td>
</tr>
<tr>
<td>10</td>
<td>Helms, Katie (VS-Sannes)</td>
<td>THE ROLE OF WNT7B AND TGF-β1 IN THE EXPRESSION OF FGF9 AND WNT5A BY HUMAN ALVEOLAR TYPE 2 CELLS AND FIBROBLASTS.</td>
</tr>
<tr>
<td>11</td>
<td>Hensley, Taylor (NS-Cheng)</td>
<td>GENERATION OF THERAPEUTIC CARDIOSPHERE-DERIVED STEM CELLS FROM ADULT CANINE HEARTS</td>
</tr>
<tr>
<td>12</td>
<td>Jin, Younggeon (GS-Blikslager)</td>
<td>LUBIPROSTONE PROTECTS AGAINST MURINE COLITIS PRINCIPALLY IN A CLC-2-DEPENDENT MANNER.</td>
</tr>
<tr>
<td>13</td>
<td>Kapatos, Alexander (GS-Hess)</td>
<td>THE TCRβ-DERIVED IDIOTYPE REGION OF NORMAL AND MALIGNANT CANINE T- CELLS CONTAIN PEPTIDES CAPABLE OF BINDING THE DLA-88*50801 ALLELE</td>
</tr>
<tr>
<td>14</td>
<td>Kim, Monica (GS-Hauck)</td>
<td>ESTABLISHING A METASTATIC SIGNATURE FOR CANINE SOFT TISSUE SARCOMAS</td>
</tr>
<tr>
<td>15</td>
<td>Knazovicky, David (NS - Lascelles)</td>
<td>EFFICACY OF CANINE ANTI-NERVE GROWTH FACTOR ANTIBODY FOR THE ALLEVIATION OF DEGENERATIVE JOINT DISEASE-ASSOCIATED PAIN IN DOGS</td>
</tr>
<tr>
<td>16</td>
<td>Kortum, Amanda (GS-Yoder)</td>
<td>DIFFERENTIAL EXPRESSION AND LIGAND BINDING INDICATE ALTERNATIVE FUNCTIONS FOR ZEBRAFISH POLYMERIC IMMUNOGLOBULIN RECEPTOR (pIgR) AND A FAMILY OF pIgR-LIKE (PIGRL) PROTEINS</td>
</tr>
<tr>
<td>17</td>
<td>Lee, Sung Hyun (GS-?)</td>
<td>DEFICIENCY OF CYCLIN D3 INCREASES THE MALIGNANT PROGRESSION OF CDK6-DEPENDENT SKIN PAPILLOMAS.</td>
</tr>
<tr>
<td>18</td>
<td>Lindquist, Danielle (VS-Smith)</td>
<td>INFLUENCE OF THREE TIMES A DAY MILKING ON HETACILLIN CONCENTRATIONS IN LACTATING DAIRY CATTLE</td>
</tr>
<tr>
<td>19</td>
<td>Loose, Rob (VS-Bailey)</td>
<td>DEVELOPMENT OF A MODEL FOR THE STUDY OF ANTIBIOTIC EFFICACY IN EQUINE UTERINE FLUID</td>
</tr>
<tr>
<td>21</td>
<td>Medland, Julia (GS-Moeser)</td>
<td>EARLY WEANING STRESS IN PIGS ALTERS THE DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM IN A SEX-SPECIFIC MANNER WHICH CONTRIBUTES TO PERSISTENT INTESTINAL BARRIER DYSFUNCTION</td>
</tr>
<tr>
<td>22</td>
<td>Miller, Adele (VS-Anderson)</td>
<td>COMPARISON OF STAPHYLOCOCCUS AUREUS FROM BOVINE AND CAPRINE MILK</td>
</tr>
<tr>
<td>Poster #</td>
<td>Author (Classification-Mentor)</td>
<td>Title</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>23</td>
<td>Milloway, Matthew (VS-Moeser)</td>
<td>CORTICOTROPIN RELEASING FACTOR RECEPTORS 1 AND 2 ARE DIFFERENTIALLY EXPRESSED IN PORCINE ILEUM AND COLON IN RESPONSE TO DIFFERENT STRESSORS</td>
</tr>
<tr>
<td>24</td>
<td>Nye, Carolyn (VS-Mariani)</td>
<td>ANALYSIS OF LACTATE CONCENTRATIONS IN THE CEREBROSPINAL FLUID OF DOGS WITH NEUROLOGIC DISEASE</td>
</tr>
<tr>
<td>25</td>
<td>Palumbo, Natalie (VS-Gadsby)</td>
<td>TUMOR NECROSIS FACTOR ALPHA REGULATES THE PROSTAGLANDIN F-2 ALPHA (FP) RECEPTOR IN CULTURED PORCINE LUTEAL CELLS.</td>
</tr>
<tr>
<td>26</td>
<td>Park, Karen (NS-Breitschwerdt)</td>
<td>REGIONAL SEROPREVALENCE OF FELINE ANAPLASMOSIS, EHRLICHIOSIS, AND BORRELIOSIS IN NORTH AMERICA AND THE CARIBBEAN</td>
</tr>
<tr>
<td>27</td>
<td>Pierce, Callie (VS-Martin)</td>
<td>ASSESSMENT OF BROILER BREEDER MORTALITY COMPARING SLAT HEIGHT AND MUSCULOSKELETAL LESIONS</td>
</tr>
<tr>
<td>28</td>
<td>Quist, Erin (GS-?)</td>
<td>PERIPHERAL BLOOD CHANGES IN RATS DEVELOPMENTALLY EXPOSED TO VOLATILE ORGANIC COMPOUNDS: SHIFTS IN LEUKOCYTE DISTRIBUTION</td>
</tr>
<tr>
<td>29</td>
<td>Ruppel, Marissa (VS-Dunning)</td>
<td>DEVELOPMENT AND VALIDATION OF A SURVEY INSTRUMENT: ASSESSING OWNER-PERCEIVED QUALITY OF LIFE IN HEALTHY DOGS</td>
</tr>
<tr>
<td>30</td>
<td>Schreeg, Megan (GS-Birkenheuer)</td>
<td>CYTAUXZOOON FELIS CYTOCHROME B PHARMACOGENOMICS: DEVELOPMENT OF A RAPID CYTOCHROME B GENOTYPING ASSAY USING HIGH RESOLUTION MELT ANALYSIS</td>
</tr>
<tr>
<td>31</td>
<td>Shelton, Molly (VS-Hanel)</td>
<td>MODE OF ACTIVATION SIGNIFICANTLY IMPACTS THROMBOELASTOGRAPHIC RESULTS AND ASSAY VARIABILITY</td>
</tr>
<tr>
<td>32</td>
<td>Sommerville, Laura (HO-Moeser)</td>
<td>NERVE AND MAST CELL INTERACTIONS IN STRESS-INDUCED GASTROINTESTINAL DISEASE: DEVELOPING A NEW CO-CULTURE MODEL</td>
</tr>
<tr>
<td>33</td>
<td>Spears, Patricia (NS-Orndorff)</td>
<td>INFLUENCE OF CELL WALL TEICHOIC ACID COMPOSITION ON LISTERIA MONOCYTOGENES CELL-TO-CELL SPREAD</td>
</tr>
<tr>
<td>34</td>
<td>Telford, Corbin (VS-Gilger)</td>
<td>SYNERGISTIC EFFECT OF ONCE DAILY TOPICAL 0.03% BIMATOPROST AND 0.5% TIMOLOL MALEATE ON INTRAOCULAR PRESSURE REDUCTION IN NORMAL BEAGLES</td>
</tr>
<tr>
<td>35</td>
<td>Thompson, Candyce (VS-Bailey)</td>
<td>AFFINITY OF LACTIC ACID PRODUCING BACTERIA TO ADHERE TO CANINE VAGINAL EPITHELIAL CELLS</td>
</tr>
<tr>
<td>36</td>
<td>Traver, Samantha (VS-Foster, Dorman)</td>
<td>OLFATORY TOXICITY IN RATS FOLLOWING MANGANESE CHLORIDE NASAL INSTILLATION</td>
</tr>
<tr>
<td>37</td>
<td>Tuohy, Joanne (HO-Lascelles, Fogle)</td>
<td>PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF DIFFERENCES BETWEEN MONOCYTES IN DOGS WITH AND WITHOUT OSTEOSARCOMA.</td>
</tr>
<tr>
<td>38</td>
<td>Turner, Rachel (VS-Gerard, Fogle)</td>
<td>RISK FACTORS FOR THE DEVELOPMENT OF INCISIONAL DRAINAGE IN 502 HORSES UNDERGOING SURGICAL TREATMENT OF COLIC</td>
</tr>
<tr>
<td>Poster #</td>
<td><strong>Author (Classification-Mentor)</strong></td>
<td><strong>Title</strong></td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>39</td>
<td>Vandergriff, Adam (GS-Cheng)</td>
<td>MAGNETIC TARGETING OF CARDIAC-DERIVED STEM CELLS WITH FDAAPPROVED FERUMOXYTOL NANOPARTICLES BOOSTS CELL ENGRAFTMENT AND THERAPEUTIC BENEFITS</td>
</tr>
<tr>
<td>40</td>
<td>Varallo, Nicole (VS-Hauck)</td>
<td>CIRCULATING LEVELS OF ANGIPOIETIN-2 AS A PROGNOSTIC MARKER FOR METASTASIS IN CANINE MALIGNANT MELANOMA</td>
</tr>
<tr>
<td>41</td>
<td>Wcisel, Dustin (GS-Yoder)</td>
<td>FUNCTIONAL AND GENOMIC CHARACTERIZATION OF NOVEL IMMUNOGLOBULIN-LIKE TRANSCRIPTS IN ZEBRAFISH</td>
</tr>
<tr>
<td>42</td>
<td>Werts, Adam (VS-Peterson)</td>
<td>INVESTIGATING THE MOLECULAR MECHANISMS OF ACTRIIB-FC-ASSOCIATED BRUNNER’S GLAND LESIONS IN MICE</td>
</tr>
<tr>
<td>43</td>
<td>Ziegler, Amanda (VS-Sannes)</td>
<td>PORCINE ALVEOLAR TYPE II CELLS AS A POTENTIAL MODEL FOR HUMAN ALVEOLAR EPITHELIUM IN CULTURE</td>
</tr>
</tbody>
</table>

VS = Veterinary Student, HO = House Officer, GS = Graduate Student
HIGHLY DIVERSE METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS (MRSP) ISOLATED FROM CANINES AT THE NCSU VHC

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North Carolina State University College of Veterinary Medicine

Staphylococcus pseudintermedius is the predominant Staphylococcus species of dogs, and is considered part of normal skin flora. On occasion, these organisms are associated with clinical disease. A subpopulation of S. pseudintermedius are resistant to methicillin (MRSP), which makes them unresponsive to treatment with β-lactam antibiotics and often multiple other drug classes. The aim of this study was to compare the antibiotic resistance profile and genetic relatedness of MRSP isolates among dogs admitted to the NCSU VHC. We hypothesized that the MRSP population would be diverse with no clear association between collection sites. Staphylococcus pseudintermedius was cultured from 297 canine patients between June 1, 2012 and May 31, 2013. Of these, 119 (40%) were MRSP. In addition to 100% β-lactam resistance, isolates commonly showed resistance to trimethoprim/sulfamethoxazole (68.9%), clindamycin (68%), erythromycin (68%), enrofloxacin (61.3%), and tetracycline (54%). Isolates from the skin represented 90% (108/119) of MRSP isolates, and exhibited a similar proportion of multidrug resistance. However, when isolates were grouped into specific skin lesions or sites, the resistance pattern varied. Pulsed-field gel electrophoresis (PFGE) was performed on 44 randomly selected isolates. Nine unique PFGE-clusters were identified with 47% similarity among all isolates. Among skin isolates, seven PFGE-clusters were identified with 61% similarity. Our results show that the genetic population of MRSPs isolated from canines at the VHC is highly diverse among site. These findings imply that there is not one standard method of antibiotic treatment for MRSP infections and individualized management based on an antimicrobial susceptibility report is necessary.
Canine cutaneous histiocytoma (CCH) is a common, benign neoplastic proliferation of histiocytes of Langerhans cell (LC) origin that often ulcerate, become secondarily infected, and regress spontaneously. *Bartonella* is a fastidious genera of intracellular pathogens that can be transmitted through arthropod bites and epidermal animal scratches and has been previously identified in the cytoplasm of histiocytes within granulomatous lesions and in skin biopsies of inflammatory pustules and papules. Based upon the established oncogenic properties of *Bartonella*, we hypothesized that *Bartonella* spp. can be molecularly detected in CCH and can be localized within skin neoplasms using indirect immunofluorescence (IIF). Paraffin embedded surgical biopsies from dogs with CCH and non-neoplastic skin adjacent to osteosarcomas (control group due to wide surgical margins) were retrieved from NCSU-CVM pathology. DNA was extracted and the 16S-23S rRNA intergenic transcribed spacer (ITS) region, pap31 and gltA genes were amplified using *Bartonella*-specific primers. IIF was performed using a *B. henselae* monoclonal or *B*. spp. polyclonal as primary antibodies and Cy3 goat anti-mouse IgG secondary antibody to localize *Bartonella* in tissues of PCR positive dogs. *Bartonella* spp. was amplified from 1/17 (5.8%) control tissues and 4/29 (13.8%) CCH tissues (p=0.63). *Bartonella* was identified in 2/4 (50.0%) CCH tissues using IIF. *Bartonella* spp. are unlikely to cause CCH. Though *Bartonella* can be visualized in CCH using IIF, cellular localization of *Bartonella* within the skin has reduced sensitivity due to low organism load, a limitation well-supported by previous attempts by our laboratory to localize the bacterium in various tissues and lesions.
IMPACT OF ENTEROCOCCUS HIRAE ON GROWTH AND INTESTINAL EPITHELIAL CELL ADHESION OF ENTEROPATHOGENIC E. COLI

Amy DiDomenico, Veterinary Student

Stephen Stauffer, Luke Borst, Mitsu Suyemoto, Jody Gookin

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NCSU CVM

Each year, thousands of kittens die with diarrhea of unknown etiology. A recent study identified that the gastrointestinal tract of healthy kittens is colonized by high numbers of enteroadherent Enterococcus hirae while enteropathogenic E. coli (EPEC) was observed exclusively in sick kittens. We hypothesize that E. hirae provides a protective advantage to intestinal epithelium either by blocking adherence of EPEC or by secreting an inhibitory substance.

To determine if E. hirae can outcompete adhesion of EPEC to intestinal epithelium, E. hirae and EPEC were used alone or concurrently to infect intestinal porcine epithelial cells (IPEC’s). After 2 hours, the monolayer was washed to remove non-adherent bacteria; all remaining material was cultured overnight to determine the number of E. hirae and EPEC adhering. Finally, strains of E. hirae from healthy kittens were investigated for the ability to cause deferred antagonism of growth of EPEC on blood agar.

Both E. hirae and EPEC were observed to adhere to IPEC’s and when inoculated concurrently, EPEC adhered in greater numbers than E. hirae. However, in the presence of E. hirae, the number of EPEC binding to the monolayer was decreased. No strains of E. hirae tested were observed to confer deferred antagonism to the growth of feline EPEC via the production of an inhibitory substance.

These studies may identify E. hirae as a competitive inhibitor of EPEC infection in young kittens and support use of E. hirae as a probiotic capable of decreasing kitten mortality.

Funding: NIH-T35 Interdisciplinary Biomedical Research Training Program; WINN Feline Foundation
MAST CELLS PLAY A PROTECTIVE ROLE IN SPONTANEOUS COLITIS

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The inflammatory bowel diseases (IBDs) are debilitating intestinal diseases that are increasing in prevalence. No cure is available and current lifelong treatment strategies carry significant side effects. Mast cells (MCs) are critical mediators of IBD but their precise role is incompletely understood. Though they have a deleterious role in acute chemical colitis models, we have recently demonstrated that MCs play a novel protective role in a spontaneous colitis model (IL10−/− mouse, C57Bl/6), as IL10−/− mice that also lack MCs (DKO:(IL-10−/− x KitWsh/Wsh) exhibited more severe colitis, diarrhea, and intestinal permeability compared with MC-sufficient IL10+/− mice. The objective of this study was to define the role of the MC in spontaneous colitis by comparing these 2 mouse strains. Colonic samples were collected for histopathology and were mounted on Ussing chambers for measurement of colonic permeability via paracellular flux of 4kDa FITC-dextran (FD4). Spontaneous colonic cytokine production was measured. Mice (DKO) were repleted with bone marrow-derived MCs (BMMCs) to demonstrate gain-of-function. DKO mice exhibited exacerbated colitis compared to IL-10+/− mice as assessed by increased colitis scores (P<0.01), IL-12p40 (P<0.05), and TNF (P<0.01). Intestinal permeability was greater in DKO mice (P<0.05). Repletion of DKO mice with BMMCs ameliorated colitis as demonstrated by reduced colitis scores, mucosal hypertrophy, TNF, and colonic permeability (P<0.05, all). These results demonstrate that MC deficiency exacerbates colitis in the IL10+/− mouse model of spontaneous IBD and highlights a novel protective role for the MC in spontaneous colitis. Further investigation of the MC’s protective mechanism may elucidate novel therapies for IBD.

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It is increasingly recognized that sex is a critical determinant in the prevalence and severity of many diseases, including irritable bowel syndrome (IBS) and IgE-mediated allergy, which are more prevalent in females than males. The disparity between sex predilection and disease has long been thought to be linked to the actions of sex hormones on immune cells; however, other potential factors such as inherent chromosomal differences or sex-specific genes have been largely overlooked. Our objective was to determine whether mast cells (MCs), a central immune cell type involved in IBS and IgE-mediated allergy, exhibit sex-specific degranulation responses to \textit{in vitro} stimuli in the absence of sex hormones. In our studies, murine female MCs released a greater percentage of their $\beta$-hexosaminidase stores than male MCs in response to calcium ionophore (26.9\% higher, p<0.001) and IgE-DNP (22.9\% higher, p<0.001). Female MCs also released significantly more histamine in response to calcium ionophore (2.3-fold higher, p<0.001) and IgE-DNP (2.9-fold higher, p<0.001) compared to male MCs. Baseline intracellular calcium and stimulus-induced calcium influx were not different between male and female MCs indicating that the sex differences were not due to differences in intracellular calcium mobilization. Taken together, these data indicate a heightened reactivity of female MCs. Future experiments will be aimed at determining whether sex-specific effects of degranulation are due to differential downstream modulation of the actin cytoskeleton. Uncovering the mechanism responsible for sex-dependent differences in MCs could allow for the development of sex-specific and more effective treatments for MC-associated diseases.

This research was supported, in part, by the following grant from the National Institutes of Health: R01 HD072968 (AJM)
Idiopathic pulmonary fibrosis (IPF) is a progressive, debilitating disease characterized by a proliferative fibrotic response and unregulated epithelial hyperplasia and metaplasia. Microscopically, the disease can be recognized by the signature histologic pattern of usual interstitial pneumonia (UIP), including honeycomb change, septal expansion, and discrete centers of myofibroblasts and their associated extracellular matrix (ECM) termed “fibroblastic foci” (FF). The normal development and expansion of ECM is in part under the control of the wingless (Wnt) family of signaling glycoproteins, many of which are up-regulated in IPF. WNT-5a, has been shown previously to promote the proliferation and resistance to apoptosis of myofibroblasts found throughout the distorted, thickened interstitium of IPF. In this study, we sought to demonstrate the histologic localization of WNT-5a in lungs of normal human and IPF lungs. Formalin-fixed and processed tissue blocks provided by the Lung Tissue Research Consortium were separated into groups based on severity of disease (normal lung, mild IPF, and severe IPF), were sectioned, deparaffinized, and processed for peroxidase immunohistochemistry, using a rabbit anti-WNT5a antibody. Results indicated that WNT-5a was confined to the cytoplasm of alveolar type 2 epithelial cells, macrophages, and mesothelial cells in normal lung tissue. However, in IPF tissue, these sites were expanded to include WNT-5a reactivity in virtually all myofibroblasts found throughout the thickened interstitium, including FF. These results demonstrate that WNT-5a is highly expressed in IPF lung tissue, and widespread in the hyperplastic and fibrotic connective tissues characteristic of the disease.
ZEBRAFISH LARVAE REVEAL A NOVEL MEDIATOR OF MACROPHAGE CHEMOTAXIS

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Tissue infiltration by macrophages and neutrophils is an early and critical step in the innate immune response. Yet excessive infiltration by these leukocytes can contribute to disease pathology. Chemotactic factors and cell surface receptors mediating leukocyte migration have been extensively studied, but the intracellular mediators of this process are less well-defined. Zebrafish (Danio rerio) are advantageous for studying innate immunity because during the first weeks of life larvae lack a functional adaptive immune response and are protected against pathogens by innate immunity. Importantly, zebrafish share many defining features of the innate immune response with mammals. We used a RNA microarray strategy to define an innate immune response transcriptome in 3-day-old zebrafish larvae exposed to a bacterial or viral mimic. Analysis of this transcriptome revealed a gene, tripartite motif containing 9 (trim9), that had increased transcript levels in response to both mimics, is highly conserved among vertebrate species and, prior to our studies, had no reported immune function. Trim9 transcript levels increase in neutrophils and macrophages of zebrafish and humans in response to toll-like receptor stimulation. Although a function for Trim9 in the immune response has not been described, its published role in control of axon growth guidance suggests it is important in cell migration. To investigate the role of Trim9 in leukocyte migration, we disrupted Trim9 function in zebrafish macrophages through expression of a dominant negative form of Trim9 (dnTrim9). We demonstrate reduced in vivo chemotaxis in zebrafish macrophages expressing dnTrim9, suggesting a novel role for Trim9 in macrophage chemotaxis.

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CHARACTERIZATION OF THE Z LINEAGE MAJOR HISTOCOMPATIBILITY
COMPLEX CLASS I GENES IN ZEBRAFISH

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Zebrafish (Danio rerio) are a valuable model for studying immunity, infection, and
hematopoietic disease and have recently been employed for transplantation assays.
However, the lack of syngeneic zebrafish creates challenges with identifying immune-
matched individuals. The MHC class I genes, which mediate allogeneic recognition in
mammals, have been grouped into three broad lineages in zebrafish: the classical U
genes on chromosome 19, the Z genes which have been reported to map to
chromosome 1, and the L genes that map to multiple loci. Transplantations between
individual zebrafish that are matched at the U locus fail to consistently engraft
suggesting additional loci contribute to allogeneic recognition. Although two full-length
zebrafish Z transcripts have been described, the genomic organization and diversity of
these genes have not been reported. Herein we define ten Z genes on chromosomes 1
and 3 and on an unplaced genomic scaffold. We report that neither of the Z transcripts
previously described match the current genome assembly and classify these transcripts
as additional gene loci. We characterize full-length transcripts for nine of these twelve
genes. We demonstrate a high level of expression variation of the Z genes between
individual zebrafish suggestive of haplotypic variation. We report low level sequence
variation for individual Z genes between individual zebrafish reflecting a possible
nonclassical function, although these molecules may still contribute to allogeneic
recognition. Finally, we present a gene nomenclature system for the Z genes consistent
with MHC nomenclature in other species and with the zebrafish gene nomenclature
guidelines.
REDUCTION IN THE NUMBER OF PROGENITOR AND STEM CELLS IN CASES OF LARGE COLON VOLVULUS

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Colic is the single-most fatal disease process in the horse. Large colon volvulus (LCV) represents one of the most severe diagnoses at a reported mortality rate ranging from 35-83%. Under normal conditions, regeneration of intestinal epithelial tissue depends on the presence of intestinal crypt stem and progenitor cells. However, little research has been done to investigate the response of these cell types to ischemic injury. The aim of this study was to determine if survival following a diagnosis of LCV is dependent on the preservation of the progenitor and stem cell zone. Pelvic flexure biopsies from clinical cases of LCV of ≥ 360° and cases in which an enterotomy was performed for non-strangulating cases of colic were fixed in formalin, paraffin-embedded, and sectioned for indirect immunofluorescence and immunohistochemistry. Antibodies against biomarkers specific to progenitor and stem cells were used to quantify differences in these cell populations between controls and LCV cases. Horses with LCV had significantly fewer progenitor/stem cells than did controls as indicated by SOX-9 and Phosphohistone-3 using one-way ANOVA (p < 0.05). Immunofluorescence data has not demonstrated number of progenitor/stem cells as a prognostic indicator but immunohistochemistry investigations are pending. Ischemic injury resulting from LCV reduces the number of crypt progenitor and stem cells in the large colon; this may one day serve as a significant prognostic indicator.

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Purpose/Objectives: “Marginal miss”, or incomplete irradiation of a targeted tumor, is clinically significant as irradiated cells can influence non-irradiated cells through bystander effects. We observed bystander effects where irradiated tumor cells triggered aggressive changes in the non-irradiated tumor. We hypothesize 1) Marginal miss will promote a more aggressive tumor phenotype in non-irradiated cells via oxidative stress leading to overexpression of hypoxia inducible factor-1 (HIF-1). HIF-1 expression will stimulate angiogenesis, epithelial mesenchymal transition (EMT), invasion/migration, and the development of metastasis. 2) These effects will be abrogated by MnBuOE, an oxidative stress inhibitor.

Materials/Methods: Partial irradiation of 4T1 tumors grown in our dorsal window chamber model (X-ray, 300 micron beam; 0, 30, 50 Gy) was performed and tumor changes were assessed longitudinally with in vivo hyperspectral imaging. 4T1 and 4T07 cells were irradiated in vitro (0, 2, 5, 10, 20Gy) and assessed for EMT via immunofluorescence for biomarkers. Cells were analyzed in a transwell assay over 24 hours +/- MnBuOE (1uM).

Results: Partial tumor radiation initiated a robust angiogenic response, infiltration of tumor cells along pre-existing vascular networks adjacent to the irradiated tumor, and migration of tumor cells to distant unirradiated sites. Immunofluorescence for biomarkers revealed a dose-responsive EMT in irradiated cells. MnBuOE reduced invasion in 4T07 cells by 2.58-fold.

Conclusions: While the project is ongoing, we have evidence that partial tumor radiation triggers aggressive tumor changes in vivo. Radiation induces EMT in 4T1 and 4T07 cells in vitro and MnBuOE can reduce the invasion in 4T07 cells.

Research supported by T32 RR024394, NCSU CMTR Pilot Grant, and RO1 NIH CA40355.
THE USE OF TRAZODONE TO REDUCE FELINE TRAVEL ANXIETY AND INCREASE VETERINARY EXAM TRACTABILITY

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Recent studies have documented a decline in the number of cats presented to veterinarians for health evaluations, in part attributed to travel distress and examination anxiety. This study evaluated the efficacy of the behavioral drug trazodone in reducing anxiety in cats when given prior to travel for veterinary examination. Trazodone is a serotonin antagonist and reuptake inhibitor that produces anxiolytic and mild sedative effects in dogs. Preliminary studies in laboratory cats suggested similar effects. We hypothesized that administration of trazodone would reduce travel anxiety and increase tractability of feline patients during the veterinary exam. In a double blind, placebo controlled, randomized crossover design, 10 cats between 1-12 years of age were recruited to the NCSU Health and Wellness Center for two examinations. Prior to travel, cats received either placebo or 50 mg trazodone per os. Results revealed that, when given trazodone compared to placebo, cats' anxiety improved significantly between home and the veterinary hospital (McNemar’s test, p=0.0156), and cats had improved ratings of tractability during veterinary examination (p=0.0313). There were no significant differences between physiologic measures of heart rate (p=0.5547) or blood pressure (p=0.9063) when given trazodone compared to placebo. Although within normal range, cats had lower respiratory rates when receiving trazodone compared to placebo (p=0.0547), which suggested a reduction in anxiety. In conclusion, the behavioral drug trazodone, given orally to cats prior to veterinary visits, decreased travel distress and examination anxiety. Trazodone may be a therapeutic option to decrease cat anxiety, promote veterinary visits, and enhance the welfare of cats.

Morris Animal Foundation Veterinary Student Scholars Program
Introduction: The purpose of this study was to evaluate the hemodynamics and sedative effects of oral transmucosal (OTM) detomidine gel and its reversal with atipamezole in dogs.

Methods: 8 adult purpose-bred dogs were utilized in a prospective study. Baseline heart rate (HR), respiratory rate (RR), cardiac output (CO; determined via lithium dilution), delivery of oxygen (DO2), systemic vascular resistance (SVR), packed cell volume (PCV), total protein (TP) and sedation score were obtained after placement of arterial and venous catheters. Detomidine hydrochloride gel (1.98±0.03 mg/m²) was administered oral-transmucosally. Hemodynamic data and sedation scores were obtained at pre-determined time points over 180 minutes. PCV/TP were taken at 0, 75, 150, and 180 minutes. Atipamezole (0.1 mg/kg) was administered intramuscularly at 150 minutes. Reversal of sedation was timed and scored. Data was analyzed using ANOVA.

Results: Compared to baseline, RR, CO, and DO2 were significantly lower, and SVR was significantly higher at 30, 45, 60, 67, 75, 82, 90, 105, 120, 135, and 150 minutes. HR was also significantly lower beginning at 45 minutes. PCV was significantly increased at 75, 150, and 180 minutes. All dogs became sedated and maximum sedation occurred at 75 minutes post administration. After atipamezole administration, total recovery time was 7.55 (±1.89) minutes and sedation was completely reversed in all dogs. No adverse events were noted.

Conclusion: OTM detomidine gel produced reliable and reversible sedation in dogs with hemodynamic effects similar to other alpha-2 agonists, and is an alternative to injectable sedatives in healthy dogs.
A COMPARATIVE EFFICACY TRIAL FOR TRIMETHOPRIM-SULFAMETHOXAZOLE, GENTAMICIN, AND PENICILLIN USING AN EX VIVO MODEL OF GESTATIONAL DISEASE

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Treatment of equine placentitis and other infectious reproductive diseases relies heavily on antibiotics. However, clinical efficacy of commonly used antibiotics is poor. In our laboratory, we developed an ex vivo model to study antibiotic efficacy under conditions characteristic of pregnancy and uterine disease.

In the current study, we aimed to test antibiotic efficacy of four antibiotics against Escherichia coli (EC) and Streptococcus equi subsp. zooepidemicus (SEZ) in Mueller Hinton broth (MHB), fetal fluids (FF) or purulent uterine fluid (PUF). Known concentrations of bacteria and two physiologically achievable concentrations (high (H)/low (L)) of trimethoprim-sulfamethoxazole (TMS), potassium penicillin (P; SEZ only), gentamicin sulfate (G), or P and G together were added to sterile autoclaved fluid. Each combination was incubated in triplicate for 8 hours, and serial dilutions were plated for quantitative assessment of bacterial load.

In PUF inoculated with EC, antibiotics were not effective, with greater than 3 log$_{10}$ growth in all samples. Bactericidal activity (3 log$_{10}$ reduction in bacterial concentration) was achieved by all antibiotics in MHB, and by either TMS or PGH in FF. Bactericidal activity against SEZ was achieved only by PGH, regardless of fluid-type. Penicillin alone (PH, PL) and PGL were bacteriostatic in all fluid-types, while TMS and G lost efficacy against SEZ (bacterial growth at 8 hours) in PUF or PUF and FF, respectively.

These findings demonstrate a profound impact of physiologic fluids on antibiotic efficacy, with PG performing best under the study-conditions. Further work to understand the mechanisms by which these antibiotics are inhibited is warranted.
USE OF A NOVEL COLLECTION METHOD TO MEASURE ACTIVE DRUG
CONCENTRATIONS IN THE GI TRACT OF CATTLE TO ASSESS RISK OF
ANTIMICROBIAL RESISTANCE IN ENTERIC BACTERIA.

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Increasing concern for the development of antimicrobial resistance in food animals, evidenced by the FDA’s restrictions on use of cephalosporins and fluoroquinolones, prompted development of a novel method to collect GI fluid using in vivo ultrafiltration probes and subsequently measure active drug concentrations for antimicrobial resistance risk assessment. Previous methods were limited to measurement of drug concentrations in fecal samples, and assessments of risk of development of antimicrobial resistance were inconclusive.

An ultrafiltration probe, which only collects low molecular weight compounds, was surgically implanted in the ileum and spiral colon of 12 Holstein steers. After surgery, 2.2 mg/kg ceftiofur sodium or 7.5 mg/kg enrofloxacin was administered per label. Blood, interstitial fluid, ileal, and spiral colon samples were collected at timed intervals for the duration of 3 drug half-lives. At the conclusion of the study, the steers were euthanized, and a full necropsy was performed.

The ultrafiltration probes were successful at extracting fluid from the GI tract, and sufficient volume to perform HPLC to measure drug concentrations was collected in 95% (114/120) of samples. Upon necropsy, the probes were still securely placed, and no significant gross pathology resulting from implantation of the probes was noted.

Ceftiofur was found in higher concentrations in the ileum, however enrofloxacin was highest in the spiral colon. Interestingly, the Cmax of enrofloxacin was greater than the plasma and peaked much later. Next, model enteric bacteria will be exposed to the drug concentrations found in the GI tract to assess the risk of antimicrobial resistance development.

Funding: CVM Intramural Grant Research Award
Little is known about the sacroiliac (SI) angle in dogs or its relationship to other angles in the pelvic limb, including the lumbosacral (LS), coxofemoral (CF), and femorotibial (FT) angles, or the pelvic tilt, and tibial-plateau slope. Variations in SI angle may affect how dogs carry their hind-limbs and thus may impact skeletal development and progression of a variety of disease states. The objectives of this study were to document the relationship of these angles within and between two dog breeds.

Images from the two most common breeds with pelvic radiographs at NCSU were retrospectively evaluated for LS and SI angles. Forty dogs from each of these breeds, were then prospectively evaluated (24 Labrador retrievers, 8 German Shepherds entered to date). SI, LS, CF and FT angles, and pelvic tilt were measured from standing horizontal beam radiographs and simultaneous photographs, and tibial-plateau slope was measured from lateral radiographs.

Retrospectively, median(range) measurements for Labradors are 148(130-167) degrees for the LS angle; and 68(48-80) and 47(30-61) degrees for the SI angle using two different methods. Prospectively, measurements for Labradors to date were 160(149-179) degrees for the LS angle; and 68(63-76) degrees for the SI angle. Tibial slope was measured on 27 dogs with interpretation pending. Shepherd data is too sparse for comment. Correlation coefficients will be generated at study end.

Conclusion: There are strikingly large within breed SI and LS differences. If correlated with other hind-limb angles, the impact of SI angle on skeletal development and disease progression will deserve further study.

Funding: Summer Research Internship Program and George H. Hitching’s New Investigator Award in Health Research
HYPOXIA AND RETROMER AS POTENTIAL REGULATORS OF POLARIZED AMYLOID PRECURSOR PROTEIN EXPRESSION IN RPE CELLS

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Amyloid precursor protein (APP) is a ubiquitously expressed protein that produces amyloid-beta (Aβ), a key component of Alzheimer’s disease and a contributing factor to age-related macular degeneration (AMD). Retromer, an intracellular retrograde transport complex, shuttles APP away from key cleavage enzymes, limiting the production of Aβ. Retromer regulates the polarized movement of APP from the trans-Golgi network to apical or basolateral surfaces for secretion. However, there is no information about the interaction and movement of APP and retromer within the retina under varied oxygen conditions. Polarized localization and secretion of APP by retinal pigmented epithelial (RPE) cells under normal and hypoxic conditions has not been studied. We have found polarized secretion of APP in RPE cells that is controlled by oxygen levels. Specifically,

- Using our unique cell culture system of polarized, tight junctional RPE cells under hypoxic conditions (0.5% oxygen) APP secretion was greatly increased in the basolateral direction
- Under hypoxic conditions, apical secretion of APP is diminished
- Hypoxia decreases retromer levels in RPE cell lysates

These results demonstrate for the first time that there is polarized secretion of APP affected by low oxygen levels. This is also the first time that hypoxia’s effect on retromer has been reported. We are currently determining if the effect of hypoxia on retromer causes alteration of the polarized secretion of APP. This is of physiological significance because Aβ is found in Drusen (present in AMD) lying under the basolateral surface of the RPE, and hypoxia is a common pathology affecting the retina.

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STIFLE ARTHROTOMY: A NEW MODEL OF SURGICAL ORTHOPEDIC PAIN IN THE DOG

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Introduction: Most models of acute postoperative orthopedic pain involve the injection of a clinically irrelevant pro-inflammatory agent. The ideal model should be clinically relevant and allow re-use of subjects. We assessed the utility of a novel model of arthrotomy postoperative pain using objectively measured limb use.

Materials and methods: Six purpose-bred beagles underwent bilateral stifle arthrotomies with a washout period between procedures. Using a randomized crossover design, each dog received placebo and a long-acting opioid (LOp). Static and dynamic ground reaction forces (GRF) were collected prior to and for 72 hours following surgery using a pressure sensitive walkway (PSW). GRF of each hind limb were compared using actual values (delta); ratios; and symmetry indices (SI). Within an individual and within a period, the difference between the measurements obtained between the control limb and limb undergoing surgery were calculated and analyzed using repeated measures ANCOVA.

Results: Significantly decreased limb use was found for placebo treatment when compared to baseline, and significantly increased limb use in the LOp group over the placebo at all times for % Body Weight Distribution [%BW_distrib], Peak Vertical Force [PVF] and Vertical Impulse [VI]. There was a significant treatment by time interaction for velocity ($P=0.03$) and all three %BW_distrib metrics ($P<0.02$). Seromas were noted in half of wounds, developing at one week following surgery.

Discussion: Overall, lameness was successfully induced over 72 hours using a novel arthrotomy model. In addition, the use of a LOp decreased lameness further confirming the validity of this model of postoperative pain.
COMPLICATION RATES ASSOCIATED WITH PERMANENT TRANSVENOUS PACEMAKER IMPLANTATION IN DOGS WITH HIGH-GRADE ATRIOVENTRICULAR BLOCK PERFORMED DURING NORMAL BUSINESS HOURS VERSUS AFTER HOURS (97 CASES, 2002-2012)

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Transvenous pacemaker implantation in dogs is associated with a relatively high complication rate. At our institution, pacemaker implantation in dogs with high-grade atrioventricular block (HG-AVB) is frequently performed as an after-hours emergency. We hypothesized that the rate of major complications would higher for dogs with HG-AVB whose pacemakers were implanted after hours (AH) compared to during normal business hours (BH). Medical records were reviewed for dogs with HG-AVB who underwent transvenous pacemaker implantation between January 2002 and December 2012 at the North Carolina State University Veterinary Teaching Hospital. Major complications occurred in 14/79 dogs (17.7%) and included pacing lead dislodgement, lead thrombosis, lead or generator migration, generator infection, and pacing failure. Incidence of major complications was significantly higher in AH dogs (10/36, 27.8%) compared to BH dogs (4/43, 9.3%) (p = 0.0411). Median survival time for all dogs was 32.5 +/- 24.7 mo. Median survival time did not differ between groups for all-cause mortality, but cardiac mortality was significantly higher in the AH group. AH dogs were younger (9.0 +/- 3.1 yr vs. 10.7 +/- 3.2 yr, p = 0.01) than BH dogs, but there were no other significant differences between BH and AH groups in terms of demographic, clinical, or procedural variables. This study demonstrates that at our institution, AH transvenous pacemaker placement is associated with a higher complication rate than BH placement. This difference may be due to a variety of human factor differences AH compared to BH.
The morphology of elasmobranch leukocytes has been well documented in the literature. However, their cytochemical staining characteristics and thus functions are still controversial, which presents a challenge in the interpretation of hemograms in these species. The objective of this study was the cytochemical characterization of leukocytes in 13 species of elasmobranchs. Blood was collected during routine physical examination from 6 shark species (sandbar shark, sandtiger shark, zebra shark, whitetip reef shark, whale shark, brown-banded bamboo shark), and 7 batoid species (smalltooth sawfish, southern stingray, manta ray, cownose ray, Atlantic ray, lesser devil ray, roughtail stingray). Blood films were evaluated after Wright and cytochemical staining for α-naphthyl butyrate esterase (NBE), chloroacetate esterase (CAE), myeloperoxidase (PER), Sudan black B (SBB), leukocyte acid phosphatase (ACP), leukocyte alkaline phosphatase (LAP), and Periodic-acid Schiff (PAS). All elasmobranch species displayed variable positivity for NBE and ACP, among granulocytes, lymphocytes, and thrombocytes. Only the sandtiger shark, Southern stingray, Atlantic ray, and Manta ray displayed CAE positivity. The only three species of the Order Orectolobiformes (zebra sharks, brown-banded bamboo sharks, whale sharks) in this study exhibited consistent positivity of PER in fine eosinophilic granulocytes and of SBB in coarse eosinophilic granulocytes. Monocytes across species were not positive for any cytochemical stain. No leukocytes were reactive to LAP. Cytochemical staining properties of elasmobranch leukocytes demonstrated great variation among species. The results of this study provide a basis for further biological and functional characterization of elasmobranch leukocytes, which will aid in the diagnostic use of hematology in these species.
Clinical trials are frequently hindered by difficulty recruiting eligible participants, increasing the timeline and limiting generalizability of results. In veterinary medicine, where proxy enrollment is required, no studies have detailed what factors influence owner participation in studies involving cats. We aimed to investigate these factors through a survey of owners at general practices.

The questionnaire was designed using feedback from a pilot study and input from clinical researchers. Owners were asked demographic questions and whether they would, would not, or were unsure about participating in a clinical trial with their cat. They then ranked the importance and influence of various factors on participation using a 5-point Likert-type scale, and incentives from most to least encouraging.

416 surveys were distributed to cat owners at 4 hospitals, two cat-only and two multi-species; 88.6% were completed. Data for importance and influence factors as well as incentive rankings were analyzed overall, by hospital type (group), location and whether owners would consider participating.

The most influential factors were trust in the organization, benefit to the cat and veterinarian recommendation. Importance and influence factors varied by willingness to participate. Ranked incentives were not significantly different across groups, with “Free Services” ranked highest.

This study provides a first look at what factors influence participation in clinical trials with cats. Given the importance placed in the recommendation of veterinarians, continued work is necessary to narrow the gap between clinical trials and general practice. The results of this study provide guidance towards improved clinical trial design, promotion and education.

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Poster Presentations
Despite proven weight loss programs, canine obesity is increasing. Characterization of transcriptional regulators of lipid metabolism in adipocytes and liver mitochondria may elucidate the metabolic balance between lipolysis and lipogenesis in obese canines. Nutrient modulation to favor lipolysis as a novel weight loss therapy is the long-term goal. The objective of this study is to characterize expression of fatty acid synthase, hormone sensitive lipase, zinc-alpha-2-glycoprotein, and TAp63 in dogs across body condition scores (BCS) and correlate these findings with body composition data and intrahepatic lipid content.

Ten dog cadavers from a local shelter were selected based on apparent health and body condition score (low-1/9-3/9; moderate-4/9-6/9; high 7/9-9/9). Blood, liver, and falciform fat samples were obtained to measure genomic expression of FAS, HSL, ZAG, and TAp63 by RNA extraction and RT-PCR. Then, dual energy x-ray absorptiometry (DEXA) scans were run to analyze body composition measures, and a liver sample was taken to measure intrahepatic lipid content.

Preliminary DEXA analysis indicates good correlation ($R^2=0.8766$) between subjective BCS and quantitative whole body adiposity. Data from subregion DEXA analysis suggests abdominal region fat (visceral + subcutaneous) is deposited at a greater rate than limb fat (subcutaneous only) as total percent body fat / BCS increases. Abdominal subregion fat and total body adiposity increase at nearly the same rate, indicating total percent body fat is as sensitive to changes in intra-abdominal fat as the abdominal sub-region used. Tissue and blood samples are being processed and RNA-stabilized for batch analysis. Liver fat analysis is in progress.

Funding: Internal Funding
Listeria monocytogenes is a food borne pathogen that has the ability to grow intracellularly and spread cell-to-cell. Listerial cell surface features required for cell-to-cell spread are incompletely documented. The L. monocytogenes glcV gene encodes a predicted glycosyltransferase (GlcV) that is necessary for the galactosylation of the repeat unit of the wall teichoic acid (WTA). Galactose addition generates the receptor site for a reference bacteriophage (phage) P35h4. GlcV mutants are phage resistant, severely attenuated in a mouse infection model, show limited cell-to-cell spread in mouse enterocyte monolayers, and become sensitive to the cephalosporin antibiotic cefotaxime. Since GlcV activity could extend to substrates besides WTA, we performed a co-reversion assay that tested the correlation between WTA and the ability to spread cell-to-cell. Starting with a glcV point mutant strain PAS590, 116 independent, spontaneously derived cefotaxime resistant (CefR) revertants were selected and screened for their reacquisition of phage sensitivity (determined in cross streak tests) and parental levels of cell-to-cell spread (determined by plaque size and morphology on mouse enterocyte monolayers). Out of 116 CefR revertants screened, 31 displayed parental levels of phage sensitivity. All of these recovered parental cell-to-cell spread characteristics and exhibited (in all cases examined by PCR) precise intragenic reversion events. All CefR revertants that remained phage resistant (49) or partially resistant (36) failed to recover parental characteristics and were (in all cases examined) conferred by extragenic reversion events. Our results support the importance of WTA galactosylation as having a crucial role in cell-to-cell spread.

Funding: Work in our laboratory is supported by Public Health Service grant R21AI103549
SEQUENCING FOUR MISMATCH REPAIR GENES IN BOXERS TO IDENTIFY MUTATIONS CONTRIBUTING TO A CANCER SUSCEPTIBILITY SYNDROME

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Boxers demonstrate a high incidence of multiple cancer types, which may be similar to cancer susceptibility syndromes in people due to mutations in DNA mismatch repair (MMR) genes. Previously, our lab found that boxers had increased numbers of single nucleotide variations (SNVs) in c-kit, an oncogene. The MMR genes are responsible for correcting single nucleotide mutations. Mutations in MMR genes, MSH-2, MLH-1, MSH-6, and PMS-2, may predispose boxers to an increased frequency of these SNVs, and some may cause cancer. This study was designed to finish sequencing MSH-2 and MLH-1 and to sequence MSH-6 and PMS-2 in their entirety to identify any mutations that contribute to a cancer susceptibility syndrome in boxers resulting from their inability to repair DNA damage.

The NCBI database was used during the primer design process. PCR techniques amplified the exons of each gene, followed by gel electrophoresis to verify the target size of each product prior to Sanger sequencing. Samples were sequenced by Genewiz and aligned and analyzed on Clone Manager and Chromas Lite to identify variations from the reference gene sequence, the published genome of canis familiaris. In silico analysis (Panther) was used to determine the probability of identified mutations causing a functional change in the proteins. If predicted to cause a functional change, the PCR product was cloned to ensure it was not a PCR introduced mutation. If functional mutations are identified in MMR genes, boxers could be screened for these mutations which could cause an increase in cancer susceptibility.

Funding: NIH T-35 Interdisciplinary Biomedical Research Training Program, Supporters to the Hauck Lab
CORTICOTROPIN RELEASING FACTOR 2 REGULATES IGE-MEDIATED MAST CELL DEGRANULATION, ANAPHYLAXIS AND INTESTINAL PERMEABILITY

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The corticotropin releasing factor (CRF) system consisting of CRF, urocortin ligands and receptors CRF₁ and CRF₂, is the primary regulator of the stress response and a key player in stress-related diseases. Our previous work has demonstrated a critical role for mast cell specific CRF receptors in stress-induced mast cell degranulation and intestinal permeability. Recently, we made the unexpected observation that mast cell CRF receptors could profoundly modulate IgE-mediated mast cell degranulation and mediator release in vitro. The objective of this study was to determine the in vivo biological significance of mast cell CRF₂ in a murine model of IgE-mediated passive systemic anaphylaxis (PSA). Mice were sensitized with anti-dinitrophenyl (DNP)-IgE overnight followed by administration of DNP-HSA antigen. CRF₂⁻/⁻ mice exhibited significantly higher serum histamine levels (p<0.05) than wildtype (WT) mice. Compared to WT mice, CRF₂⁻/⁻ mice exhibited enhanced intestinal permeability as determined by increased FITC dextran (4 kDa) permeability across jejunal (41% increase; p<0.05) and colonic mucosa (160% increase; p<0.05) and a parallel reduction in TER. To confirm the role of mast cell CRF₂ in IgE-mediated anaphylaxis, mast cell deficient mice (Kit⁺⁺⁻⁻) were engrafted with WT or CRF₂⁻/⁻ bone marrow derived mast cells followed by induction of PSA. Mice engrafted with CRF₂⁻/⁻ mast cells responded with higher serum histamine and greater reduction in body temperature to PSA than mice repleted with wildtype mast cells. Together, these results indicate that mast cell CRF₂ plays a novel role in regulating IgE-mediated mast cell degranulation, intestinal injury, and anaphylaxis.
Enterococcal Spondylitis (ES) outbreaks are responsible for significant losses to the broiler chicken industry with mortality ranging from 4-20%. ES is caused by genetically related, pathogenic strains of *Enterococcus cecorum* containing putative virulence genes. The study of these virulence genes requires a rapid, inexpensive bioassay for *E. cecorum* virulence. We assessed the ability of a chick embryo lethality test to correctly identify virulent and avirulent field isolates of *E. cecorum*. We hypothesized that 12 day old chick embryos inoculated with virulent strains would have higher mortality than those inoculated with commensal cecal strains. Fertilized broiler chicken eggs and layer (specific pathogen free) eggs were used in this study. At day 12 of incubation, the allantoic cavity was inoculated with 100ul of bacteria. An initial dose-response experiment over $10^0$ to $10^8$ cfu was used to optimize dose for both broiler and SPF eggs. A dose of $10^2$cfu/egg was used for all subsequent injections. Mock (no inoculum) and PBS inoculation served as controls. Embryo survival was assessed by candling. Kaplan-Meier product limit estimates of survival curves were used to compare strain differences with the log rank test used for significance testing. Isolates recovered from spinal abscesses proved to be significantly ($p<0.001$) more virulent compared to cecal isolates in both broiler and SPF eggs. This egg embryo lethality test is a good model for differentiating between virulence phenotypes and will be useful for screening for attenuation of future gene deletion mutants.
THE RIPPLING EFFECTS OF ECOTOXICOLOGICAL ENDOCRINE DISRUPTING COMPOUNDS ON FISH IN NORTH CAROLINA WATER SYSTEMS

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Currently, there is a knowledge gap pertaining to the effects that endocrine disrupting compounds (EDCs) have on fish populations in North Carolina water systems. It has been well documented that contaminant EDCs, such as estrogenic compounds, leech into waterways from myriad sources, including agricultural effluents, disposed pharmaceuticals, industrial emissions and pollutants, urban wastewater, and residential runoff. Previous studies have evaluated the deleterious effects of these EDCs on fish reproductive and immune systems, and this investigation served to build upon these pertinent findings relative to the North Carolina waterways. This study aimed to develop a more complete understanding of the effects of EDC related impacts on wild fish species in North Carolina rivers and streams, venturing to create a definitive relationship among exposure, reproductive, endocrinological, and immunological impairments in fish. Utilizing immunoassays and histopathology on field-sampled fish tissues, we garnered evidence to support theories of estrogen-mediated immunosuppression and reproductive endocrinopathies. Additionally, histopathological examination revealed parasitic infiltration in many of the harvested samples, fostering inquiries about the plausibility of parasite-mediated immunosuppression, gonadal atrophy, or physiological impairment. This research is critical because waters adulterated by effluent EDCs can have individual and population impacts on fish, damage essential aquatic ecosystems, detrimentally affect revenues the state generates from fisheries, and ultimately taint food supplies for wildlife and humans. Future studies may constitute upon this work to amass a deeper knowledge of carcinogenesis and endocrinopathies, potentiating the need for developing relevant aquatic animal models of human disease via experimental bioassays.

Funding source: Extramural grant from the North Carolina Wildlife Research Council, and contract funds.
Acknowledgement: North Carolina State University College of Veterinary Medicine, Merial Veterinary Scholars Program.
Occupational exposure to inhaled manganese (Mn) is associated with altered odor recognition (dysosmia); however, the dose response relationship between acute Mn exposure and dysosmia remains poorly understood. This study evaluated whether intranasal instillation of Mn (as MnCl$_2$) impairs a rat’s ability to discriminate between two odors previously learned in a ‘Go/No-Go’ odor discrimination task (OD). Nine 10-week-old Fischer 344 rats were trained using operant techniques to discriminate between vanillin (rewarded conditioned stimulus - CS+) and amyl acetate (unrewarded conditioned stimulus - CS-). Once the OD task was mastered, rats (n= 4-5 rats/group) were anesthetized with isoflurane and a bilateral 40 μl intranasal instillation of either 200 mM MnCl$_2$ or 0.9% saline (vehicle control) was administered. Rats were retested 48 hours later using the vanillin/amyl acetate OD task. The rats were then euthanized and olfactory epithelium and olfactory bulbs collected from each rat to measure tissue Mn levels. The rats learned to discriminate between amyl acetate and vanillin and accurately (>80% correct responses) perform a Go/No-Go OD task within 20.8 days±8.5 (mean±standard deviation) of task introduction. Manganese exposed rats had increased olfactory epithelial and olfactory bulb Mn concentrations and impaired OD performance when compared with saline-treated controls. Future studies can better define the relationships between olfactory bulb Mn concentration and odor discrimination in rodents thereby further demonstrating the value of our rodent nasal instillation model as a surrogate for Mn inhalation.

Funding: Merial Veterinary Scholars Program and an unrestricted gift from Afton Chemical Corporation
Detection of clinically relevant pain relief in cats with degenerative joint disease (DJD) has been complicated by a profound placebo-by-proxy effect. Worsening clinical signs following withdrawal of medication is often discussed as a clinical phenomenon, but to date, has not been included in clinical trial design.

Deterioration of clinical signs following withdrawal of active medication was assessed in a double-masked, placebo-controlled study. Cats with DJD-associated pain and mobility impairment received active treatment (meloxicam) or placebo for 21 days followed by a masked washout period of 21 days (placebo administered). Improvement during treatment as well as a return of clinical signs (deterioration) during the washout were evaluated using two owner-completed clinical metrology instruments (CMI: Feline Musculoskeletal Pain Index, FMPI; Client Specific Outcome Measures, CSOM) and objective accelerometry data.

Fifty-eight cats were assessed. After the first treatment period, both meloxicam and placebo treated cats showed significant improvement (p<0.0001) on both CMIs, but no significant difference between the groups. Following washout, cats that had received meloxicam had a greater return of clinical signs than the placebo treated cats (CSOM: p=0.048; FMPI: p=0.021). Cats receiving meloxicam had higher activity (p<0.0001) than the placebo group during the treatment period and showed a significant decrease in activity during washout (p=0.019) such that there was no difference in activity between groups during the washout period (p=0.938).

The placebo-by-proxy effect complicates detection of efficacy over placebo in analgesic treatment trials. However, these data suggest a novel trial design method for mitigating this effect - the masked washout period.

Funding: Study funding and AT salary support were received from a grant from Boehringer Ingelheim (553396); MG was supported by NIH T32 (OD011130).
RESPONSE OF EQUINE CHORIOALLANTOIS TO INFLAMMATORY STIMULI AND ANTI-INFLAMMATORY DRUGS

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Intrauterine inflammation due to bacterial infection is a major cause of pregnancy loss in mares. Mares rapidly eliminate compromised pregnancies, and this process is likely precipitated and mediated by prostaglandins secreted by the uterus or conceptus. Thus, treatment with anti-inflammatory drugs represents a key aspect of clinical management of high-risk equine pregnancies.

In this study, we hypothesized that fetal and embryonic chorioallantoic tissue would respond to lipopolysaccharide (LPS, 1µg/mL) or heat-killed Streptococcus zooepidemicus (kSz, 5x10⁷ CFU, term tissue only) via an increased production of prostaglandins PGF2α and PGE2. Further, we hypothesized that co-culture with pentoxifylline (PTX, 10 uM) or flunixin meglumine (FM, 10uM) would inhibit prostaglandin production. Chorioallantois from four near-term mares and ten 33-34 day embryos were pre-treated with ibuprofen (10mM) for one hour to diminish effects of harvesting and then cultured with media alone, or in the presence of LPS, kSz, PTX, FM or a combination of these treatments for 24 hours. The supernatants were analyzed for PGF2α and PGE2 using commercial ELISA (Cayman, Inc).

Fetal tissues treated with LPS or kSz increased prostaglandin production compared to control and addition of FM inhibited this increased prostaglandin production, but PTX did not (PGF2α p=0.0008, PGE2 p=0.0024 and PGF2α p>0.1, PGE2 p>0.1, respectively). Embryonic tissues had numerical responses to LPS (p>0.05) and FM significantly diminished PGF2α and PGE2, compared to LPS-treated samples (PGF2α p=0.0003, PGE2 p=0.0001), whereas PTX did not. These findings suggest the conceptus is an active participant in inflammation and support the use of NSAIDs to prevent abortion.

Research Support: NCSU-CVM Internal Research Grant, 2012-2013

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The role of Wnt7B and TGF-β1 in the expression of FGF9 and Wnt5A by human alveolar type 2 cells and fibroblasts.

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The goal of this study was to model specific cell-cell interaction between fibroblasts and epithelial cells that reflect those that might occur in pulmonary alveolar repair following injury, like observed in idiopathic pulmonary fibrosis. Human primary type II alveolar cells (hAT2) were co-cultured with human lung fibroblasts (HLF), with or without transduced overexpress Wnt7B by one or the other, and with/without treatment with 20ng/mL of TGF-β1. Cells were harvested at 48hr for qRT-PCR for FGF-9 and Wnt5A, two potentially important mediators of fibrogenic processes in the lung. HLFs alone or transduced with Wnt7B in culture with 20ng/mL TGF-β1 had an increase in FGF-9 expression. When HLFs were co-cultured with hAT2 with or without Wnt7B overexpression, a dramatic decrease in FGF-9 expression was observed, regardless of TGF-β1 addition. HLFs transduced with Wnt7B in co-culture with hAT2 also demonstrated an increased Wnt5A expression, which was further increased with addition of TGFβ1. hAT2 cells alone or transduced with Wnt7B in culture with TGF-β1 also had an increase in FGF9 expression. But when co-cultured with HLFs with or without transduced Wnt7B overexpression with TGF-β1 treatment, FGF9 was less, but remained above control levels. hAT2 cells alone or transduced to overexpress Wnt7B and treated with TGF-β1 had increased Wnt5A. But this effect on Wnt5A in the hAT2 cells was blunted by co-culture, regardless of Wnt7B overexpression. These results suggest that FGF9 is up-regulated by TGF-β1 in both cells whereas Wnt5A is up-regulated by Wnt7B in hAT2 cells and by both Wnt7B and TGF-β1 in HLFs.

Funding: HL-4497-20
Background:
Cardiosphere-derived cells (CDCs) have been shown to reduce scarring, increase viable myocardium, and boost cardiac function after myocardial infarction in preclinical animal models and a recently completed human trial (CADUCEUS). The hypothesis tested is that similar to previous models, canine CDCs could be harvested and expanded from dog hearts and these cells should exhibit phenotypic and functional similarity to human CDCs.

Method/Results:
Canine CDCs (cCDCs) are grown from endomyocardial tissue samples. Collagenase digestion yields cardiac progenitor cells from myocardial tissue. The morphology of these cells is similar to that from human and rodent CDCs. cCDCs can undergo >20 doubling in <30 days. Such growth potential facilitates cell manufacturing for either autologous or allogeneic applications. Cell colonies could be derived from a single cCDC. The antigenic profile of cCDCs is similar to that of human CDCs as they express CD105 (>90%) and c-kit (~5%) and do not express markers such as CD90, CD45, CD31, and CD34.

Conclusions:
Our data establishes the identity and manufacturing feasibility of cCDCs as a unique cardiac stem cell population derived from adult hearts. These findings, when tied to previous research, shows this process is ready to advance to the next step to deliver cCDCs into dogs with heart failure to achieve therapeutic regeneration.

Funding Sources: NCSU Chancellor’s Faculty Excellence Program (CFEP), American Heart Association.
Lubiprostone Protects Against Murine Colitis Principally in a CIC-2-Dependent Manner.

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Lubiprostone, a CIC-2 chloride channel activator, induces intestinal secretion and is used in the management of idiopathic chronic constipation. However, we have previously reported that lubiprostone initiates intestinal barrier repair in ischemic-injured intestine via its principal target, the chloride channel CIC-2. Thus, we hypothesized that lubiprostone would have a protective effect in an in vivo colitis model. We administrated lubiprostone in a dextran sulfate sodium (DSS)-induced colitis model in wild type and CIC-2\(^{-/-}\) mice. We determined the severity of colitis based on body weight, disease activity index (DAI), histology scores, and levels of cytokine production. Administration of DSS in the drinking water initiated symptoms of experimental IBD, including weight loss and elevation in DAI. Oral administration of lubiprostone protected against weight loss and significantly reduced DAI (P<0.05) in a dose-dependent manner. In addition, lubiprostone significantly reduced histology scores, and colon shortening as compared to DSS-treated wild type mice that were not pre-treated with lubiprostone. When administered to CIC-2\(^{-/-}\) mice, lubiprostone treatment had a limited protective effect against DSS colitis. Specifically, loss of body weight, DAI, and intestinal permeability were significantly reduced in the high dose lubiprostone treatment group of CIC-2\(^{-/-}\) mice, but they showed no difference in colon length and histology score. In conclusion, lubiprostone has a major protective effect in a CIC-2 dependent manner in experimental colitis model, but it also has some alternative protective mechanisms of action at high dosages. Additional investigation is required to determine the detailed mechanisms of action of lubiprostone in experimental colitis model.

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THE TCRβ-DERIVED IDIOTYPE REGION OF NORMAL AND MALIGNANT CANINE T-CELLS CONTAIN PEPTIDES CAPABLE OF BINDING THE DLA-88*50801 ALLELE

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Abstract:
The inevitable development of chemoresistance to lymphoma condemns most patients and alternative treatments are needed if survivals are to improve; antigen-specific immunotherapy is a potential means. For this purpose, malignant TCR idiotypes (ID) could be an important antigen with low tolerance. Vaccination in human lymphoma patients can elicit anti-ID antibodies, but clinical responses are poor. CD8+ cytotoxic T-lymphocytes (CTLs) are potent anti-tumor effectors, yet it is unclear whether CTLs which can recognize the ID antigen of malignant T cells exist. We hypothesize that such CTLs exist and can be primed to kill malignant (or auto-reactive) lymphocytes. While such CTLs are yet to be demonstrated in canines, the presence of ID-derived peptides capable of presentation in class I molecules would provide strong circumstantial evidence that such cells may exist. To find these potential peptides, we sequenced the ID (CDR3) region of the TCR β chain from 7 canine T-cell lymphoma nodal biopsies. Previously, we have successfully performed an in silico peptide binding prediction for DLA-88*50801, a prevalent canine class I allele, using HLA-A*6801 as a model. In this study we found that out of 7 malignant and 41 non-malignant published ID sequences, several contain 1 or more putative binders. This work suggests that the existence of an anti-TCR CTLs is possible. It follows that such cells could be expanded by immunotherapeutic means to control T-cell lymphoma or T-cell related autoimmune diseases.
Despite improvements in our ability to control a tumor locally, metastatic disease remains the most common cause of cancer-related death. Accurate identification of patients likely to develop metastatic disease is important for early treatment, and prevents unnecessary treatment of patients who will not develop metastasis. In particular, while most soft-tissue sarcomas (STS) can be effectively treated locally with current therapy, identification of tumors that will metastasize remains highly inaccurate. A better understanding of the genetic changes responsible for metastasis would allow more accurate prognoses and identify novel therapeutic targets. Our study aimed to identify such differentially expressed genes and proteins.

We compared differentially expressed genetic and proteomic data from canine metastatic and non-metastatic STS samples. Pathway analysis and data merging was performed between genetic and proteomic expression data to discover common findings. In the group of overlapping protein/gene expression, we found 3 proteins that were higher in metastatic tumors (DCN, GAPDH, and GPI) and 1 protein that was higher in non-metastatic tumors (MAP1B). These proteins were further investigated using PubMed, IHOP, Summon, and additional online resources. GAPDH and GPI play critical roles in glycolysis, and extracellular GPI promotes cell motility. MAP1B has been implicated in promoting cell death via senescence.

Four differentially expressed proteins have been identified between metastatic and non-metastatic STS data, with two additional proteins (ACTR2 and HNRNPD) demonstrating varying isoform expression. Mapping patterns of differential expression may be prognostic for sarcomas, such as predicting metastasis, and holds promise in identifying targets for novel therapeutic approaches.
Degenerative joint disease (DJD) is the most common cause of chronic pain in dogs. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the only approved class of drugs for DJD pain in dogs. Antibodies against Nerve Growth Factor (NGF) are analgesic in rodent models and in humans with DJD. The aim of this pilot study was to evaluate the efficacy of a novel caninised anti-NGF antibody (NV-01) for the treatment of DJD pain in dogs.

In a placebo-controlled, masked clinical study, 26 dogs with DJD were randomized to NV-01 (200 mcg/kg IV) or placebo. Owners completed clinical metrology instruments (Client-Specific Outcome Measures [CSOM] and Canine Brief Pain Inventory [CBPI]) on days 0, 14 and 28. CSOM and CBPI subscales (pain severity [PS] and pain interference [PI]) were evaluated within and between groups non-parametrically. Recognized success/failure criteria were applied and success compared between groups using Fisher’s exact test.

CBPI PS and PI scores did not change in the placebo group, but significantly improved in the NV-01 group (PS: Day 0-14, p=0.010 and Day 0-28, p=0.025; PI: Day 0-14, p=0.006 and Day 0-28, p=0.032). CSOM scores showed a similar pattern of improvement, and indicate a significant difference between groups at day 14 and day 28 (p=0.011 and p=0.033, respectively), and significantly more successes at day 28 (p = 0.037; Chi-Square). No side effects were noted.

These data demonstrate a positive analgesic effect of anti-NGF antibody in dogs suffering from chronic pain. The magnitude of the effect was identical to that expected with an NSAID.

Funding source: Nexvet Biopharma Pty Ltd
DIFFERENTIAL EXPRESSION AND LIGAND BINDING INDICATE ALTERNATIVE FUNCTIONS FOR ZEBRAFISH POLYMERIC IMMUNOGLOBULIN RECEPTOR (pIgR) AND A FAMILY OF pIgR-LIKE (PIGRL) PROTEINS

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The polymeric immunoglobulin (Ig) receptor (pIgR) is an integral transmembrane glycoprotein that plays an important role in the mammalian immune response by transporting soluble polymeric Iggs across mucosal epithelial cells. Single pIgR genes, which are expressed in lymphoid organs including mucosal tissues, have been identified in several teleost species. A single pigr gene has been identified on zebrafish chromosome 2 along with a large multigene family consisting of 29 pigr-like (PIGRL) genes. Full length transcripts from 10 different PIGRL genes that encode secreted and putative inhibitory membrane bound receptors have been characterized. Although PIGRL and pigr transcripts are detected in immune tissues, only PIGRL transcripts can be detected in lymphoid and myeloid cells. In contrast to pIgR which binds Iggs, certain PIGRL proteins bind phospholipids. PIGRL transcript levels are increased after infection with Streptococcus iniae, suggesting a role for PIGRL genes during bacterial challenge. Transcript levels of PIGRL genes are decreased after infection with Snakehead rhabdovirus, suggesting that viral infection may suppress PIGRL function.

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DEFICIENCY OF CYCLIN D3 INCREASES THE MALIGNANT PROGRESSION OF CDK6-DEPENDENT SKIN PAPILLOMAS.

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The Cyclin-Dependent Kinases 4 and 6 (CDKs) belong to a family of serine-threonine kinases that are cell cycle regulated by association with D-type cyclins at specific time-points of the cell cycle. We have previously established that overexpression of CDK6 in mouse epidermis (K5CDK6) lead to reduction of the multiplicity and incidence of skin tumors. Our previous results have shown that CDK6 preferentially binds to cyclin D3. Thus, we hypothesized that the CDK6/cyclin D3 complex negatively impacts skin tumorigenesis. To test this idea, we developed K5CDK6/Cyclin D3−/− mutant mice and subjected them to a carcinogenesis protocol. Contrary to our hypothesis, lack of cyclin D3 expression does not affect the tumor repression activity of CDK6 since both, K5CDK6 and K5CDK6/D3−/− mice, exhibit a severe reduction of the number of skin tumors. However, histopathological analysis revealed that 60% of K5CDK6/D3−/− tumors were classified as squamous-cell carcinomas with a variable degree of squamous differentiation. Biochemical analysis of epidermal tumors show that cyclin D3 ablation results in a rise of cyclin D1 level, suggesting that cyclin D3 does not affect the tumor suppressive role of CDK6, but the increased level of cyclinD1 elevates the rate of malignant progression.

We conclude that CDK6 activity can prevent tumor formation during the initial stages of tumorigenesis in a cyclin D3-independent manner. However, lack of cyclin D3 alters the susceptibility of CDK6-expressing tumors to malignant conversion. Therefore, therapies directed to reduce D-type cyclins expression should be considered within the context of the expression level of CDK6 and CDK4.

Supported by NIH grant CA116328.
Clinical mastitis in dairy cattle is commonly treated with intramammary antibiotics. The purpose of this study was to determine whether once a day dosing of hetacillin is sufficient to maintain therapeutic drug concentrations in milk for a 24-hour period in cows that are being milked three times per day. After collecting a baseline milk sample, 8 Holstein cows received intramammary infusions of hetacillin in the left front and right rear quarters once a day for 3 days, following label instructions. Each cow was milked 3 times per day throughout the study period. Composite samples representing milk from each of the treated quarters were collected at each milking for a total of 6 days and frozen until analysis. Milk ampicillin concentrations were determined using UPLC-MS/MS. Average concentrations of ampicillin in milk in both quarters were 6.98 + 1.50, 0.48 + 0.25, and 0.025 + 0.005 µg/mL at 8, 16 and 24 hours after intramammary infusion. These data indicated ampicillin concentrations exceed the MIC for most mastitis pathogens for 16 hours, but not for the full 24-hour dosing interval in this study. In dairy cattle milked three times per day, it would be recommended to infuse intramammary hetacillin every 16 hours to maintain adequate drug concentration in the mammary gland.

Funding: Boehringer Ingelheim Vetmedica
Equine endometritis and postpartum metritis are associated with excess intrauterine fluid and bacterial overgrowth, commonly *Escherichia coli* (EC) and *Streptococcus zooepidemicus* (SZ). These conditions are treated with antibiotics, but treatment failure can occur. This manuscript describes a novel *ex vivo* method of testing to identify antibiotics that retain efficacy in uterine fluid. We hypothesized that EC and SZ would logarithmically replicate in uterine fluid collected from normal postpartum mares or mares with pyometra, and the addition of either ceftiofur or trimethoprim sulfamethoxazole (TMS) would inhibit bacterial growth.

Sterile uterine fluid obtained <12 hours postpartum (PPS), autoclaved uterine fluid (PPA) and autoclaved material from a mare with pyometra (PYO) were inoculated with EC ($2.60 \times 10^7$ CFU/mL) or SZ ($9.50 \times 10^6$ CFU/mL). Samples were coincubated with ceftiofur (2mg/mL), trimethoprim (0.55mg/mL) and sulfamethoxazole (7.4 mg/mL) or no antibiotic for 8 hours. Serial ten-fold dilutions were plated on Columbia Agar to determine bacterial load of samples. Different fluid-types and treatments were compared using ANOVA.

*Escherichia coli* and SZ grew in all inoculated fluids. Antibiotics resulted in approximately 1000-fold inhibition in CFU-counts in PPS and PPA fluids ($p=0.009$) relative to control. Ceftiofur-treatment resulted in approximately 1000-fold inhibition in CFU-counts in PYO fluid ($p=0.04$) relative to control, whereas TMS-treated samples were not different from control CFU counts in PYO fluid ($p>0.1$) relative to control.

This study established an *ex vivo* model for the study of antibiotic activity in uterine fluid and demonstrated that efficacy of ceftiofur in all fluid-types, while TMS was ineffective in purulent uterine fluid.

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Merial Summer Research Program
MEASUREMENT OF ACTIVE ANTIBIOTIC CONCENTRATIONS IN THE PULMONARY EPITHELIAL LINING FLUID AND INTERSTITIAL FLUID OF HEALTHY CALVES

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Bovine respiratory disease is the biggest health challenge facing the beef cattle industry today, accounting for 75% of illnesses and 50% of deaths on feedlots. The purpose of this study is to determine the drug concentrations produced at the site of infection, the pulmonary epithelial lining fluid (PELF), and the correlation between PELF and interstitial fluid (ISF) when calves are given an antibiotic.

Twenty-four 200kg steers were administered either tulathromycin (n=6), florfenicol (n=6), ceftiofur crystalline free acid (n=6), or enrofloxacin (n=6) per label instructions. PELF, ISF, and plasma were collected for at least 3 half-lives. PELF was collected via a novel naso-tracheal intubation method in which absorbent cotton was passed through a flexible tube to collect the sample in a minimally invasive manner. Using this method, we were able to collect a sufficient amount and quality of fluid to be analyzed without causing any pathologic changes in the animal, thus eliminating the need for euthanasia. ISF and PELF concentrations along with in vitro assays of protein binding and lipophilicity were compared to determine the impact of drug properties on diffusion into the airways.

The drug concentrations in plasma, ISF, and PELF were analyzed via HPLC, followed by pharmacokinetic analysis. ISF and PELF concentrations along with in vitro assays of protein binding and lipophilicity will be compared to determine the impact of drug properties on drug diffusion into the airways. Observed drug concentrations in the PELF will be compared to the documented MIC’s for Mannheimia hemolytica to assess each drug’s effectiveness against the common bovine respiratory pathogen.

Funded by Bayer Animal Health
Early life stress is linked to GI diseases during adulthood in humans (e.g. IBS, IBD) and in animals. Our previous studies demonstrated that pigs subjected to early weaning stress (EWS) exhibited long-lasting defects in intestinal barrier function that mirror the pathophysiology of IBS; however, the mechanism remains poorly understood. Our aim for this study was to determine whether EWS in pigs alters the enteric nervous system (ENS) and if ENS changes contributed to persistent, elevated intestinal permeability. Piglets were randomly assigned to two experimental groups: EWS (15 d weaning age, n=6) and late-weaned controls (LWC; 28 d weaning age, n=6). At two months post-weaning, ileum was harvested to measure intestinal permeability on Ussing chambers and analyze the ENS via immunofluorescence. Compared with LWC pigs, EWS pigs exhibited increased ileal permeability, determined by increased (p<0.5) flux of 4 kDa FITC Dextran (FD4). Permeability was particularly high in EWS females compared with EWS males and, the addition of muscarinic antagonist, atropine, reduced permeability. EWS pigs also showed increased enteric innervation, determined by PGP9.5 staining (17.1±1.82 and 10.84±0.95 neurons/field for males and females, respectively, compared with 3.58±0.47 and 9.07±0.71 neurons/field for LWC counterparts). Females exhibited a 30% higher proportion of cholinergic neurons compared with males, determined by choline acetyltransferase (ChAT) staining. EWS also resulted in a modest increase in the percentage of ChAT-positive neurons in both males and females. These data indicate that EWS results in alterations in development of the ENS, which may contribute to intestinal barrier disturbances in a sex-specific manner.

Funding Source: National Institutes of Health
Staphylococcus aureus is a frequent cause of infection in humans and animals. It is the most common cause of chronic subclinical bovine mastitis. Genotypes of S. aureus associated with one host can be transmitted among species. The purpose of this study was to compare genotypes and antimicrobial susceptibilities of representative bovine and caprine S. aureus isolates, and determine possible interspecies transfer of genotypes. It was hypothesized that genotypes and antibiotic susceptibility patterns of S. aureus would be similar for bovine and caprine isolates.

Isolates of S. aureus included 32 caprine milks submitted to the Mastitis and Milk Quality Laboratory for diagnostics, and 60 bovine milks representative of the laboratory’s database. Antimicrobial susceptibilities were determined against twelve antibiotics and genotypes were identified using pulsed field gel electrophoresis following Smal or Cfr9I digest. Genotypes were considered of the same group if they were ≥ 80% similar. Testing by PCR for the mecA gene and specific DNA sequences was performed to identify methicillin resistant S. aureus (MRSA) and/or ST398, more commonly isolated from pigs, poultry and humans.

A total of 13 genotype groups were identified, with the proportion of common genotype groups (4/13) not differing from those specific to caprine dairies (3/13), and to bovine dairies (6/13). More caprine (9/32) than bovine (2/60) isolates were resistant to three or more antibiotics. ST398 was identified, as were resistant genotypes identical or closely related to human CDC MRSA strains, showing potential trans-infection from humans and other animals. The presence of resistant strains requires further study.
CORTICOTROPIN RELEASING FACTOR RECEPTORS 1 AND 2 ARE DIFFERENTIALLY EXPRESSED IN PORCINE ILEUM AND COLON IN RESPONSE TO DIFFERENT STRESSORS

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There is increasing evidence that stress plays a role in human and animal gastrointestinal disorders. While it is known that the corticotropin releasing factor system plays a role in diseases such as Irritable bowel syndrome, the mechanism of the stress response in regulating other stress modes such as microbial insult or psychosocial stress has yet to be determined.

This study was designed to evaluate the expression of corticotropin releasing hormone receptors 1 and 2 (CRF₁ and CRF₂) in the porcine intestine when subjected to various stressors.

Mucosal scrapings from porcine ileum were harvested from control pigs and pigs subjected to different stressors including: infectious stress (salmonella infection) and social mixing stress. Protein was extracted and concentration was determined by BCA assay. Protein samples were subjected to gel electrophoresis. Western blotting was performed with antibodies specific for CRF₁ and CRF₂. The gels were then stripped and reprobed for GAPDH as a loading control.

Densitometric analysis of western blots was performed and compared between stressors. Expression of CRF₁ and CRF₂ was observed to be differentially expressed between the modes of stress. In the social mixing stress model, CRF₁ exhibited a five-fold increase in expression compared to control samples. When subjected to S. typhimurium insult, CRF₂ was up-regulated with a two-fold increase compared to controls.

These results indicate a role for the CRF system in more global responses to diseases outside the realm of psychosocial stress. The up-regulation of these receptors also suggests the potential of these receptors for therapeutic targeting in treatment of GI disease.

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ANALYSIS OF LACTATE CONCENTRATIONS IN THE CEREBROSPINAL FLUID OF DOGS WITH NEUROLOGIC DISEASE

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Funding sources: Merck-Merial, Department of Clinical Sciences Start-up funds

Lactate is a product of cellular metabolism. It is produced in higher quantities in anaerobic conditions than aerobic. Therefore, blood lactate is commonly used in the emergency room and critical care facilities as a sensitive indicator of tissue perfusion. It correlates directly with disease severity and inversely with prognosis. Previous human studies have shown increases in CSF lactate with primary and metastatic CNS neoplasia, and comparatively higher increases with meningitis. This study was designed to determine the feasibility of analyzing CSF lactate with a “cageside” monitor (the Lactate Plus) as well as the effect of different storage conditions on lactate concentrations. In addition, the utility of CSF lactate as a biomarker for differentiation of neoplastic versus inflammatory CNS disease was assessed.

CSF lactate was measured in 63 dogs that presented to the NCSU Neurology Service for diagnostic evaluation. Twenty-five samples were analyzed immediately after collection while 38 samples previously stored at -80°C were thawed and evaluated. Repeatability of the meter was demonstrated, as was stability of lactate in CSF when stored at -20°C or -80°C for one week. Lactate was evaluated in CSF from 20 dogs with CNS inflammatory disease, 22 with CNS neoplasia and 16 others, and 5 controls. Mean CSF lactate values were 2.89, 2.26, 2.05 and 1.79 in these groups respectively. The highest values (7, 6.4) were seen with inflammatory cases; however, there was no statistically significant difference in the lactate values between these groups.
TUMOR NECROSIS FACTOR ALPHA REGULATES THE PROSTAGLANDIN F-2 ALPHA (FP) RECEPTOR IN CULTURED PORCINE LUTEAL CELLS.

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Prostaglandin F-2α (PGF-2α) is critical to induce luteolysis at the end of the porcine estrous cycle. This luteolytic action of PGF-2α is mediated by the PGF-2α (FP) receptors, which increase from day 10 or 13. While the control of FP is unknown, the number of macrophages infiltrating the corpus luteum and secretion of tumor necrosis factor-α (TNF-α) increases on days 7-18, compared to days 4-6. Thus, the hypothesis examined is that TNF-α will preferentially increase the expression of FP receptors in porcine luteal cells, increasing the binding of PGF-2α to induce luteolysis. Luteal cells were isolated from porcine ovaries collected during the early and mid-stages (approximately days 4-6 and 7-12, respectively) of the estrous cycle, and cultured (D.0). Luteal cells were treated with 0 ng/ml, 1 ng/ml, or 10 ng/ml TNF-α concentrations beginning on culture D.1. On D.3, RNA and/or protein were extracted using standard procedures. mRNA steady-state levels were examined by RT-qPCR using primers targeting porcine FP. Protein analysis of FP receptor was carried out by Western blot using antibody recognizing porcine FP. RT-qPCR results showed a marked dose-dependent decrease of the FP receptor mRNA in luteal cells collected from early and mid-cycle CLs. In contrast, FP protein levels showed a dose-dependent increase in response to TNF-α treatment in luteal cells from early stage CLs, but little change in mid-cycle stages. TNF-α has profound effects on FP receptor expression, suggesting that TNF-α may play an important role in regulating FP receptors in the porcine CL during the estrous cycle.

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Vector-borne diseases are a major concern in companion animals. While numerous studies have been conducted to better understand the exposure risks in dogs, there is little information regarding cats. To address this need, we utilized an experimental rapid, diagnostic SNAP® Multi-Analyte Assay (SNAP® M-A) from IDEXX Laboratories (Westbrook, Maine) that contains a set of species-specific peptides to detect antibodies to *Ehrlichia canis* (Ec), *E.chaffeensis* (Ech), *E.ewingii* (Eew), *Anaplasma phagocytophilum* (Aph), *A.platys* (Apl) and *Borrelia burgdorferi* (Bb). Feline serum (n=684), archived from 2008 to 2013 at NCSU-CVM Vector-Borne Disease Diagnostic Laboratory, were tested. Of the 684 samples, 202 (38.3%) were from the US South, followed by the Northeast (26.8%), Midwest (16.1%), MidAtlantic (8.6%), and 6.0% from the West. In addition, there were 26 (3.8%) samples from Canada and 3 (0.4%) from Caribbean islands.

Overall, 8% were exposed to a vector-borne pathogen. *B.burgdorferi* (5.7%) was the most common exposure, most prevalent in the Northeast and MidAtlantic regions (3.8% and 1.46% respectively). *A.phagocytophilum* (1.9% overall) followed with 1.32% seroprevalence in the Northeast. Of note is that all *Ehrlichia* spp commonly found in dogs also appear to infect cats. We conclude that increased availability of diagnostic testing will facilitate more accurate documentation of vector-borne pathogen exposure in cats.
Lameness in broiler breeders is a frequent cause of breeding soundness failure and a source of increased morbidity and mortality. Lameness morbidity and mortality is attributable to increased persecution and decreased access to feed and water. In broiler breeder hens, lameness is associated with a decrease in egg production and poor feed conversion. Lameness in roosters can cause drops in fertility due to their inability to effectively mount and inseminate hens. There are a number of factors believed to contribute to broiler breeder lameness including slat height. Slat height may be overlooked because other contributing factors can distort the clinical assessment of a problem flock. In this study, the parameters evaluated include slat height, breed, weight, flightiness, age, flock mortality, and house management. During flock assessments, dead and cull birds were necropsied to determine the cause of death with a focus on the musculoskeletal system. Data is still in the process of being analyzed to determine if there is a correlation between musculoskeletal pathology and slat height as well as other management parameters.

Funding for the project was graciously provided by the North Carolina Breeder and Hatchery Association. Funding for technical support was also provided by the CVM Veterinary Scholars Program.
Volatile organic compounds (VOCs) such as benzene, trichloroethylene (TCE), 1,2-dichloroethylene (1,2-DCE), perchloroethylene (PERC) and vinyl chloride (VC) are important industrial solvents used in dry cleaning agents, dyes, and varnishes, among others. Since the mid-1970s, environmental or occupational exposures to these VOCs have been associated with increased incidences of cancer, particularly leukemia and lymphoma. Epidemiological studies indicate that ingestion of contaminated drinking water or inhalation of volatile gases in the air, are the most likely routes of environmental VOC exposure. Developmental exposures to VOCs (e.g. during pregnancy or in infancy) can be especially detrimental and often result in malformations, congenital abnormalities and later life disease. In this study, Harlan Sprague-Dawley rats were pre-natally and peri-natally exposed to drinking water containing a VOC-mixture of 5, 10 and 50-fold well water concentrations (5X, 10X, 50X) of benzene, TCE, DCE, PERC and VC from gestation day 12 (GD 12) through post-natal day 48 (PND 48). While all dosed animals exhibited elevated leukocyte counts compared to age-matched controls, 5-7-fold elevations were observed within the two higher dose groups (10X and 50X). In addition, examination of peripheral blood smears of the 10X and 50X animals revealed increased numbers of circulating large granular lymphocytes, particularly within the 10X group. These changes possibly suggest a bone marrow response in leukocyte production or, more likely, a shift in leukocyte distribution from the tissue reservoir to the circulatory pool as a result of developmental exposure to VOCs.

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The objective of this study was to develop and validate a survey to evaluate owner-perceived quality of life (QOL) in healthy canine models in order to provide a tool for future studies with canine cancer patients.

A comprehensive literature review was performed evaluating QOL in companion animals to determine the important components to include within a QOL survey. A QOL survey tool was then developed which focused on the pet’s overall health, pain and mobility, and behavioral preferences. The survey consisted of three parts: a structured questionnaire score using a five-point Likert scale, a weighted quality of life score using a visual-analogue scale and an overall QOL assessment score using a visual-analogue scale. Survey feedback was obtained from veterinary students and clinicians through cognitive interviewing of subjects to further improve the survey’s accuracy and readability. The correlation among the three scores was used to determine the internal validity of the survey, as well as a comparison of which method of questioning is most accurate in determining owner-assessed QOL in dogs.

Results of this study show that this survey can be an accurate and reliable tool used to obtain owner-perceived QOL in healthy dogs, and the structured questionnaire was the most precise measurement of QOL. In the future this survey can be used in a trial with canine cancer patients to determine if exercise rehabilitation improves canine QOL and should be incorporated as a part of cancer treatment.
Cytauxzoon felis is a virulent tick-transmitted protozoan parasite that infects felines. Without treatment, as few as 3% of infected domestic cats survive. Treatment combining atovaquone and azithromycin (A&A) has increased the survival rate to 60%. Atovaquone treatment targets C. felis cytochrome b (cytb). Recent work done by our lab has identified an association between a C. felis cytb genotype (cytb1) and survival when treated with atovaquone and azithromycin. We hypothesized that by using real-time PCR and high-resolution melt analysis we would be able to distinguish C. felis cytb1 from other cytochrome b genotypes. DNA samples were available from 69 cats with cytauxzoonosis; all samples’ C. felis cytb genotypes had been previously characterized by DNA sequencing. PCR assays were designed to identify single-nucleotide polymorphisms (SNPs) in the C. felis cytb gene that distinguish cytb1 from other cytb genotypes. The resulting amplicons were analyzed using high-resolution melt analysis. By assessing high resolution melt clustering at five different SNP sites, 100% of the cytb1 samples were accurately identified. This test can rapidly provide prognostic information for clients considering atovaquone and azithromycin treatment in cats with cytauxzoonosis.

Funding for this study was provided by an anonymous donor; stipend support for MES was provided by the Triangle Community Foundation.
The objective of this prospective study was to evaluate the impact of mode of activation on thromboelastography (TEG). Forty-eight client-owned dogs deemed healthy by physical examination, complete blood count, serum chemistry and coagulation panel were included. TEG was performed by a single operator on citrated whole blood using four activators: kaolin, tissue factor (TF) diluted 1:3600, TF diluted 1:50000, and a TF/kaolin mixture.

Interassay variability was assessed using all four activators in a subset of eight dogs analyzed on four separate occasions. Intraassay variability was evaluated by repetitive evaluation of a single activator on four separate channels analyzed on two separate occasions. For all assays, five TEG variables were analyzed: reaction time (R), clotting time (K), alpha angle (α), maximum amplitude (MA), and global clot strength (G).

Overall differences in TEG variables were analyzed with repeated measures ANOVA, using the Tukey-Kramer adjustment for multiple comparisons. Significant differences between modes of activation were found for all variables (p<0.05). Interassay and intraassay evaluations showed that samples activated by kaolin and TF at a 1:3600 dilution had the lowest degree of variability, and the lowest coefficients of variation (<10%) were detected with α and MA. The results of this study suggest that the use of strong activators and more reliance on MA and α in interpretation may improve the utility of TEG in clinical practice.

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NERVE AND MAST CELL INTERACTIONS IN STRESS-INDUCED GASTROINTESTINAL DISEASE: DEVELOPING A NEW CO-CULTURE MODEL

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Stress-induced gastrointestinal (GI) disorders including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are chronic, incurable, and dramatically reduce the quality of life. Symptom severity and Initial onset depend on central and peripheral activation of the corticotropin releasing factor (CRF) system. We previously showed that CRF induces mast cell degranulation by activating the mast cell-specific CRF receptors CRF1 and CRF2. CRF-induced mast cell degranulation triggers intestinal permeability, a central pathogenic process mediating downstream diarrhea and abdominal pain. Neurotransmitters modulate mast cell degranulation via direct interaction with enteric nerves. Recently, utilizing a porcine intestinal model for CRF-induced intestinal mast cell degranulation and intestinal permeability, we showed that neuronal input is crucial for CRF-induced mast cell degranulation and epithelial barrier dysfunction. Further characterization of this relationship requires development of a nerve and mast cell in vitro co-culture model. For this we utilized the rat adrenal gland pheochromocytoma PC-12 cell line and the rat basophilic leukemia RBL-2H3 mast cell line. PC-12 cells cultured with nerve growth factor differentiate into neurons with sympathetic and parasympathetic character. In preliminary experiments we demonstrated that while CRF had no effect on RBL-2H3 cell degranulation, RBL-2H3 cells cultured with differentiated PC-12 cells release an average of 2200% more (n=6, p<0.001) beta hexosaminidase in response to CRF. A simplified model of nerve and mast cell communication can help characterize molecular and signaling mechanisms of the mast cell-specific CRF system, which could potentially increase our fundamental understanding of stress-induced GI disease and lead to discovery of novel therapeutic targets.
INFLUENCE OF CELL WALL TEICHOIC ACID COMPOSITION ON *LISTERIA MONOCYTOGENES* CELL-TO-CELL SPREAD

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*Listeria monocytogenes* is a rod shaped gram-positive bacterial pathogen that causes listeriosis. A crucial listerial virulence trait is its ability to replicate within cells of the liver and spleen and to spread from cell-to-cell without exposure to the host’s immune system. Our laboratory has been interested in how listerial cell surface features influence the ability of listeriae to spread cell-to-cell. We recently identified two novel genes (*glcV* and *pmpA*) whose products are required for listerial cell-to-cell spread in mouse enterocyte monolayers. Interestingly, the cell wall teichoic acid (WTA) composition in *pmpA* and *glcV* mutants is also altered. WTA is a class of highly substituted glycopolymers that are attached to the peptidoglycan cell wall of gram positive microorganisms. We have employed a systematic approach to eliminate specific WTA carbohydrate substituents through mutation and assayed each mutant’s ability to invade and spread from cell-to-cell in cultured mouse enterocyte monolayers. We find that specific WTA substituents are crucial for cell-to-cell spread. In our best-studied WTA mutant, that lacks the galactose substituent, electron micrographic evidence suggests that WTA structure is altered. Additionally, fluorescence microscopy images suggest that the normal unipolar distribution of ActA, a surface molecule required for actin based intracellular motility, appears to be bipolar in the WTA mutant. This symmetrical distribution of ActA in the mutant may reduce motility due to the requirement for asymmetric actin polymerization to propel the bacterium from cell-to-cell.

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SYNERGISTIC EFFECT OF ONCE DAILY TOPICAL 0.03% BIMATOPROST AND 0.5% TIMOLOL MALEATE ON INTRAOCULAR PRESSURE REDUCTION IN NORMAL BEAGLES

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Funding provided by Allergan, Inc. – manufacturer of Lumigan® (Bimatoprost 0.03%)

Introduction: Glaucoma is characterized by an increase in intraocular pressure (IOP), leading to optic nerve damage and rapid blindness. Recent research suggests that acute IOP fluctuations also contribute to axonal damage. The purpose of this study was to examine the efficacy of topical Bimatoprost 0.03% and Timolol 0.5%, as monotherapies and in combination, to limit IOP diurnal variation in dogs.

Methods: Untreated control IOPs were measured (via Tonovet® tonometer) in nine normotensive, adult, male laboratory beagles at 0, 2, 4, 8 and 24-hours post-administration daily for three days (short term; n=5) and once daily on days 3, 7, 10, and 14 of the treatment period (long term; n=4). The IOP schedule was repeated after a single daily dose (short term) or twice daily dose (long term) of 0.5% Timolol, and a single daily dose of 0.03% Bimatoprost or both medications (unfixed combination, UFC) for three days (short term) or fourteen days (long term), consecutively.

Results: In the short term study, lowest daily mean IOP was 14.70 ±1.21mmHg (Timolol); 11.40 ±0.97mmHg (Bimatoprost); 11.55 ±1.75mmHg (UFC); and 14.70 ±1.59mmHg (Control). Only Bimatoprost and UFC significantly reduced IOP from Control - UFC by day one (p=0.016) and Bimatoprost by day two (0.040) of treatment. Mean IOP over 14 days was 15.75 ±1.99mmHg (Timolol); 14.00 ±1.77mmHg (Bimatoprost); and 11.56 ±1.83mmHg (UFC).

Conclusions: Once-daily Bimatoprost-Timolol combination therapy resulted in a more rapid reduction of IOP compared to Bimatoprost or Timolol monotherapy. This synergistic IOP-lowering effect of Bimatoprost and Timolol supports further clinical evaluation of this combination in glaucomatous patients.
AFFINITY OF LACTIC ACID PRODUCING BACTERIA TO ADHERE TO CANINE VAGINAL EPITHELIAL CELLS

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Lactic acid producing bacteria (LAB) have been shown to prevent growth of pathogenic organisms of the human vagina. One mechanism by which LAB achieve this is through competitive displacement of other organisms on vaginal epithelial cells. This study was designed to determine the affinity of 7 strains of LAB to adhere to canine vaginal epithelial cells (CVEC).

Lactic acid producing bacteria (Weisella sp, 2xLactobacillus spp., 4xEnterococcus spp.) obtained from vaginal swabs of 7 healthy bitches were incubated in MRS broth for 24 hours. Cells obtained from 3 healthy bitches were washed in citric acid-Na2HPO4 buffer at a pH of either 7 (mimicking canine vaginal pH) or 4.3 (human vaginal pH). A suspension of 1x10^6 CVEC/mL and 1x10^8 LAB/mL, confirmed via direct hemocytometer count, was incubated for 1 hour at 37°C and 5%CO2 at a pH of 7 or 4.3 and with or without agitation (200rpm). CVEC were then collected by filtration (10μm filter) and washed with ice-cold buffer, removing non-adherent bacteria. Bacteria adhered to 26 cells from each sample were counted under light microscopy from stained impression smears. Bacterial counts were compared between treatments using an ANOVA with Repeated Measures test.

Bacterial adherence was suppressed at the lower pH (7.6±0.4 vs. 15.5±0.9; p=0.002); and was lower in non-agitated samples (12.4±0.8 vs. 18.6±1.6; p=0.01). Adherence of two organisms (Weisella sp. and Enterococcus caninestinti, strain 1) was significantly higher than the control (27±6 vs. 16±3; p=0.009). These organisms may have probiotic activity in the canine vagina.

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Following inhalation, Mn travels along the olfactory nerve from the olfactory epithelium (OE) to the olfactory bulb (OB), and exposure has been associated with altered odor recognition (dysosmia). This study evaluates two hypotheses: (a) intranasal Mn administration increases OE and OB Mn concentrations; and (b) intranasal Mn exposure impairs performance of rats trained on a go-no-go olfactory discrimination (OD) task. Rats were trained to lever press (“go”) in response to a positive conditioned stimulus (CS+: vanillin) or to do nothing (“no go”) when presented a negative conditioned stimulus (CS-: amyl acetate). Following training, rats were assigned to Mn (200 mM MnCl$_2$) or 0.9% saline treatment group (n = 4-5 rats/group). Intranasal administration of either saline or MnCl$_2$ was performed on isoflurane-anesthetized rats as 40 µL injections using PE10 tubing. Rats were retested 48 hours later using the OD task and euthanized by CO$_2$ inhalation, followed by collection of the OE and OB. Tissue Mn concentrations were analyzed by ICP-MS. Another cohort of rats (n = 3-4/group) was euthanized 48 hours after nasal instillation procedures for histopathological examination of nasal tissues. Mn-exposed rats had increased Mn levels in the OE and OB and decreased performance in the OD task when compared with controls. Histopathological evaluation of the caudal nasal cavity showed moderate suppurative inflammation of the olfactory epithelium and submucosa of the ethmoid turbinates and suppurative exudate in the nasal sinuses in MnCl$_2$ dosed animals. These newly developed nasal instillation and OD procedures are useful methods to assess Mn-induced olfactory deficits.

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The purpose of this study was to identify phenotypic and functional differences between peripheral monocytes from untreated dogs with osteosarcoma (OSA) and age-matched controls.

Ficoll centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs). The PBMCs were stained with antibodies against CD14, CD32, CD62L, CD16, CD11c, CCR2, CCR7, CD43, CX3CR1, and CXCR2 and analyzed using flow cytometry. In OSA dogs (n=18), surface expression of CD16 was increased, and expression of CD62L, CCR2, CCR7, CD43,CX3CR1 and CXCR2 was significantly decreased, compared to control (n=13) dogs, (p<0.01 for all receptors). There was a trend towards higher peripheral blood monocyte counts in OSA (n=18) compared to control (n=10) dogs.

For functional studies, monocytes were isolated from PBMCs using high speed cell sorting. Half of the cells were stimulated with LPS for 6 hours. Reverse transcription rT-PCR was used to assess TNFα, IL-10, IL-12, and COX-2 mRNA expression, and culture supernatant PGE₂ levels were assessed by ELISA. No significant difference was noted in relative mRNA expression. PGE₂ secretion was higher in OSA (n=8) compared to control (n=8) dogs (p=0.04).

These results indicate a down-regulation of monocyte chemokine receptors in OSA-bearing dogs. We postulate that monocytes are sequestered in the peripheral blood of OSA dogs, unable to migrate to tumor sites. PGE₂ has been reported to suppress T-cell responses. We postulate that increased PGE₂ secretion in OSA-bearing dogs leads to immunosuppression and debilitation of anti-tumor activity. Further work should evaluate chemotaxis in monocytes, and T-cell function of control and untreated OSA dogs.

Funding: This project was supported by the AKC Canine Health Foundation.
Colic, or gastrointestinal disease, is the number one cause of death for horses in the United States. Up to 10% of colic cases require surgical intervention, and 18 to 37% of postoperative colic patients have incisional complications. Incisional complications increase patient morbidity and treatment costs, and may affect the horse’s chance of returning to performance. The aim of this study was to determine risk factors for development of incisional drainage following exploratory celiotomy for colic in horses referred to NCSU. Horses were included in the study population if they had an exploratory celiotomy for gastrointestinal disease and survived greater than 24 hours post-operatively. Incisional drainage was defined as serous, serosanguinous, or purulent discharge persisting or starting greater than 24 hours post-operatively. Pre-operative, intra-operative, and post-operative data were obtained from the North Carolina Colic and Digestive Disease Program database over the years 2003 to 2013. The data were organized and coded using Microsoft Excel and JMP, and statistically analyzed with logistic regression using SigmaPlot 12. Of 650 surgical colic patients 502 survived greater than 24 hours. Incisional drainage developed in 61/502 (12.2%) horses. The use of an abdominal bandage postoperatively was a significant protective factor (95% CI 0.22-0.82) and shaving the abdomen with a razor preoperatively was a significant risk factor (95% CI 1.04-3.93) for the development of incisional drainage. Identifying risk factors for incisional complications may help modify surgical techniques and perioperative therapies to reduce the incidence of incisional complications and improve the prognosis following surgery.

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MAGNETIC TARGETING OF CARDIAC-DERIVED STEM CELLS WITH FDA-APPROVED FERUMOXYTOL NANOPARTICLES BOOSTS CELL ENGRAFTMENT AND THERAPEUTIC BENEFITS

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Stem cell transplantation is a promising therapeutic strategy for acute or chronic ischemic cardiomyopathy. A major limitation to efficacy in cell transplantation is the low efficiency of retention and engraftment, due at least partially to significant early “wash-out” of cells from coronary blood flow and heart contraction. Here we load cardiosphere-derived cells (CDCs) with FDA-approved ferumoxytol nanoparticles Feraheme® (Fh). Labeling efficiency is confirmed by transmission electron microscopy. In vitro assays indicate Fh labeling is nontoxic to CDCs. Fh-labeled rat CDCs (FH-rCDCs) were intracoronarily infused into syngeneic rats, with and without magnetic targeting. Magnetic resonance imaging, fluorescence imaging, quantitative PCR revealed magnetic targeting increased cardiac retention of transplanted Fh-rCDCs. Neither infusion of Fh-rCDCs nor magnetic targeting exacerbated cardiac inflammation or caused iron overload. The augmentation of acute cell retention translated into more attenuated left ventricular remodeling and larger therapeutic benefit (ejection fraction) at 3 weeks after treatment. Histology revealed increased cell engraftment and angiogenesis in hearts from the magnetic targeting group. Fh labeling facilitates magnetically-targeted stem cell delivery into the heart and may also provide noninvasive monitoring of implanted cells in clinical trials.

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This study seeks to determine if circulating levels of Angiopoietin-2 (Ang-2) are prognostic for metastasis in canine malignant melanoma. Autocrine stimulation of melanoma tumor cells by Ang-2 is important for tumor cell migration and invasion. Serum Ang-2 concentrations are prognostic for metastasis in human melanoma patients. Preliminary work in our lab yielded a statistical difference in serum Ang-2 concentrations in metastatic compared to non-metastatic canine melanoma patients.

Serum concentrations of Ang-2 from 17 patients with melanoma and 17 age, breed, and size matched controls are measured using a Novateinbio canine Ang-2 sandwich ELISA kit. Of the 17 patients with melanoma, 8 patients metastasized within four months of diagnosis, and 5 patients were not recorded to have metastasized.

The concentration of Ang-2 in the supernatant of 6 melanoma tumor cell lines are also measured using the canine Ang-2 ELISA kit. The cells were incubated with serum-free media for 24 hours before the media was centrifuged, and the supernatant harvested. Each of the groups are compared using an unpaired Student's t-test with a Welch’s Correction.

Canine patients with melanoma showed significantly higher levels of Ang-2 than their healthy age and size matched controls, $p=0.0212$. There was no difference seen between metastatic and non-metastatic patients, $p=0.1660$. There was also no correlation between Ang-2 levels and time to metastasis, $p=0.9033$.

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FUNCTIONAL AND GENOMIC CHARACTERIZATION OF NOVEL IMMUNOGLOBULIN-LIKE TRANSCRIPTS IN ZEBRAFISH

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The immunoglobulin (Ig) superfamily (IgSF) includes membrane bound and secreted proteins that possess one or more Ig domains that share core conserved residues and structural features with immunoglobulins (e.g. antibodies). Numerous IgSF members play significant roles in immune function. Hundreds of IgSF members have been identified within genomes of various teleost fish species. For example, novel Ig-like transcripts (NILTs) that encode membrane bound proteins possessing one or two Ig domains have been identified in carp, salmon, and trout. Although NILTs can encode either activating or inhibitory signaling motifs, their cellular function remains unknown. Three predicted genes encoding NILTs were previously described on chromosome 1 of the model species, zebrafish (Danio rerio). Data mining the surrounding genomic sequence of the predicted NILT genes revealed a cluster of >100 uncharacterized immunoglobulin domains phylogenetically related to NILTs that spanned ~1.2 Mbp of DNA. We have sequenced transcripts corresponding to 21 of these genes and are actively cloning additional transcripts. Our sequence data suggests polymorphic and haplotypic variation for these genes. We are pursuing the targeted genomic disruption of select members of this gene cluster. Once stable zebrafish lines are generated, we can assess the relevance of these genes to immune function with in vivo assays. Ascertaining what role these genes play in zebrafish immunity will shed important light onto their role in all teleost fish, including aquaculture species, and is a direct aim of our research.

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INVESTIGATING THE MOLECULAR MECHANISMS OF ACTRIIB-FC-
ASSOCIATED BRUNNER’S GLAND LESIONS IN MICE

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Cancer cachexia involves muscle wasting, increased fatigue, and is associated with a diminished quality of life and increased mortality. Currently, there is no FDA approved treatment for this condition. In experimental models of cancer cachexia, the TGF\textbeta superfamily ligands Myostatin and Activin A, which both inhibit growth and differentiation of muscle, are over expressed. We have developed molecules that bind and inhibit these TGF\textbeta ligands, with the goal of targeting cancer cachexia and related conditions. As a positive control for this drug development, we have used a fusion protein consisting of a solubilized TGF\textbeta receptor, ACTRII-B, connected to the Fc portion of an antibody, called ACTRIIB-Fc. This fusion protein leads to increased muscle mass under experimental conditions, however, significant side effects result: choroid plexus thickening and hemorrhage, nail bed angiectasis and hemorrhage, and atrophy of the Brünner’s Glands with hyperplasia of the overlying epithelia. Since these changes are significantly adverse, we wanted to follow up on the pathogenesis caused by ACTRIIB-Fc in order to avoid targets that could cause such lesions in the future. Guided by gene expression changes in the Brünner’s Gland tissue in response to ACTRIIB-Fc, we found that among others, Wnt5a, a secreted glycoprotein involved in cell fate decisions, as well as angiopoietin-2 (Angp-2), a secreted ligand involved in angiogenesis, were up regulated. These results, combined with further \textit{in vitro} experimentation, suggest that ACTRIIB-Fc is capable of altering cell fate in the Brünner’s Gland and interfering with blood vessel development at multiple locations in the mouse.
The development of an animal cellular model for human alveolar epithelium could greatly accelerate the pace for experimental results in the study of lung diseases. Porcine models have become valuable resources for human research and medicine. The primary barrier to the use of porcine alveolar type 2 cells (AT2s) in pure culture as a model for human AT2s is the inability to efficiently remove contaminating fibroblasts from mixed porcine lung cell isolates. Previous studies have shown that cis-hydroxyproline at a concentration of 100μg/ml effectively depletes fibroblasts from mixed cell cultures of human, chicken and mouse lung cell preparations. With this mind, we conducted a preliminary study in which 25, 50, 100, and 200μg/ml cis-hydroxyproline were added to the culture medium of primary porcine lung fibroblasts to determine the concentration required for fibroblast depletion. 200μg/ml was found to be required, and was used to treat both porcine and human primary AT2s not depleted of fibroblasts. Daily photographs were taken of the cell populations and the cells were lysed at day 2 and day 4 to be analyzed by Western blot for phenotypic protein signatures of AT2 cells and fibroblasts to determine the dominant cellular phenotype. Visualization of the cultured cells revealed that the cis-hydroxyproline inhibits proliferation and decreases viability of all porcine cell types, while selectively inhibiting only fibroblasts from the human cells. The conclusions of this study are that cis-hydroxyproline is not a useful method for depleting mixed porcine isolated lung cells of fibroblasts, and that further study comparing porcine and human AT2 physiology is required to assess the validity of the model.

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