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In forensic casework, animal DNA particularly those from companion animals is often collected as evidence. Complications can arise during DNA analysis when the sample is a mixture of more than one individual and may not be analyzed or be deemed as inconclusive. For example, in a dogfighting case investigators might find blood on the fur of a dog that could include its own blood but also that of its canine attacker. For such mixtures, probabilistic genotyping software could have potential for identifying the number of individual sources and their corresponding genetic profiles. The goal of this study is to assess whether probabilistic genotyping software can be used to separate canine DNA mixtures, such that they could be interpreted in forensic casework. In this study, 80 canine samples from NC State’s Vector Borne Disease laboratory were obtained from "bully breeds" commonly associated with dog fighting cases or breeds used in police work. The Dogfiler panel, designed for canine individualization by UC Davis’s Veterinary Genetics Laboratory, was used to obtain genotypes for each sample. Using this panel, genotype information for 13 of the 15 loci was obtained. Artificial DNA mixtures made from two individuals at varying ratios (1:1, 1:2, 1:3, and 1:4) were also genotyped with DogFiler and were analyzed using probabilistic genotyping software MaSTR (SoftGenetics, State College, PA). We will present results on the challenges of using DogFiler for canine genotyping and preliminary findings on the use of MaSTR for mixture separation.
Women experience increased susceptibility to fear-based psychiatric conditions—including anxiety and post-traumatic stress disorders—during their reproductively viable years, suggesting a relationship between ovarian hormone fluctuations and dysregulation of emotional memory. Animal models demonstrate that ovarian hormone fluctuations across the estrous cycle profoundly impact neuronal function and synaptic plasticity throughout the brain, indicating a role for hormonal states in modulating emotional memory. Therefore, we sought to investigate the state-dependent effects of estrous cycle on emotional memory using the well-established model of Pavlovian threat conditioning. Male and female mice underwent cued threat conditioning and a few days later underwent cued threat memory recall, with the experiment timed such that females were either in proestrus (when ovarian hormones levels are highest) or diestrus (when ovarian hormone levels are lowest) stages for both behavioral testing days (proestrus>proestrus, diestrus>diestrus) or such that they were in the opposite estrous stage for each testing day (proestrus>diestrus, diestrus>proestrus). Results demonstrate overall sex differences and state-dependent impacts of ovarian hormones in female threat memory acquisition and recall. Next, we sought to identify brain circuitry involved in these sex- and estrous-specific effects on emotional memory. Whole brain c-Fos analysis was performed following cued threat conditioning or naive treatment in males, proestrus females, and diestrus females. Results reveal sex and hormone differences in activation of both canonical and non-canonical brain regions associated with threat memory. Together, findings demonstrate strong influences of ovarian hormones in modulating female threat memory behavior and suggest novel female-specific emotional memory brain circuitry.
Chronic obstructive pulmonary disease (COPD) is a common inflammatory disease that restricts airflow to the lung. It often arises as a result of prolonged exposure to particulates such as those from cigarette smoke and is characterized by inflammation of the bronchial tubes (chronic bronchitis), increased mucus production, and damage to the alveoli (emphysema). There are few treatments available to slow down the disease progression and none yet able to reverse its effects. As the therapeutic benefit of lung spheroid cell-derived exosomes (LSC-Exo) was shown in pulmonary fibrosis murine models, we next analyzed the therapeutic effects of LSC-Exos on transgenic COPD murine models. LSC-Exos and PBS were delivered via nebulization treatments for seven consecutive days at a dose of 10 billion particles/kg. It was anticipated that the LSC-Exos would repair the alveoli, thus increasing lung function in the COPD murine models. Lung function was measured using spirometry tests, including the forced expiratory volume (FEV)/forced vital capacity (FVC) ratio. Initially, COPD mice had FEV/FVC ratios significantly below that of their wild-type counterparts, and treatment with LSC-Exos was found to increase that ratio. Additionally, mice receiving LSC-Exo treatment showed a decrease in airway wall thickness and mucin-producing cells, via H&E and PAS staining respectively. LSC-Exos rescue lung function by reducing airway thickness and mucin production in COPD murine models.
DEVELOPING A THROMBOELASTOGRAPHY ASSAY IN ELASMOBRANCHS

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North Carolina State University College of Veterinary Medicine and Center for Marine Sciences and Technology

Abstract: Thromboelastography (TEG) is a viscoelastic hemostatic assay evaluating clot initiation time, kinetics, and strength, as well as the extent of fibrinolysis in whole blood or plasma. TEG has been thoroughly studied in mammals but has been used less extensively in non-mammalian species such as elasmobranchs. Elasmobranchs, a subclass containing sharks, rays, and skates, are often found in aquarium collections with wild populations currently declining. Non-traumatic coagulopathies often go unnoticed in exhibit animals until advanced stage disease has developed posing a challenge to providing definitive or palliative care. The study goal was to establish a TEG protocol in elasmobranchs that may improve detection of hemostatic defects and expand therapeutic options. Pooled samples of citrated plasma from wild caught and aquarium elasmobranchs were stored at -80°C until day of use. TEG was performed using multiple clotting initiators, specifically RapidTEG (tissue factor with kaolin), kaolin, Reptilase, and elasmobranch-derived thromboplastin. Initial tests yielded no quantifiable TEG reactions with any of the initiators investigated. TEG analyses may have been negatively impacted by the naturally high plasma calcium concentration of elasmobranchs, or the brain-derived thromboplastin may have been inactivated in preparation due to low urea concentration. In contrast, TEG analyses using fresh whole blood and fresh plasma samples led to measurable TEG reactions with multiple clotting initiators. Further work is needed to determine the most reliable clotting initiator for elasmobranchs, the effects of fresh vs. frozen and pooled vs. individual samples, and establish normal reference ranges.

Funding sources: Support Fund for Aquatic Animal Medicine (NC Veterinary Medical Foundation account), NC State CVM Veterinary Scholars Program

Primary subject category: Clinical medicine
EFFECT OF IN OVO VACCINATION WITH HERPESVIRUS OF TURKEY SUPPLEMENTED WITH TOLL-LIKE RECEPTOR 3 AGONIST (POLY I:C) ON INNATE AND CELL-MEDIATED IMMUNITY IN MEAT-TYPE CHICKENS

A. Boone1,2: graduate student
R. R. Kulkarni1, A. Cortes1, T. Villalobos3, J. Esandi3, I. Gimeno1
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1NCSU CVM, 2Rollins Animal Disease Diagnostic Laboratory, 3Zoetis-Global Biodevice

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We have demonstrated that in ovo vaccination of herpesvirus of turkey (HVT) hastens the immunocompetence of 1-day-old meat-type chickens by accelerating the maturation of innate and cell-mediated immunity. This positive effect was optimized by modifying the vaccine dose. The recommended dose (RD, 6080 plaque forming units, PFU) had the best immunopotentiating effects compared to half-dose (3040 PFU), quarter-dose (1520 PFU) and double-dose (12160 PFU). The objective of this study was to evaluate if adjuvantation of HVT with a Toll-like receptor 3 agonist, polyinosinic-polycytidylic acid (poly (I:C)), could enhance vaccine-induced responses and provide a dose-sparing effect. The HVT vaccine was given at half the RD supplemented with 50µg poly (I:C) [HVT-1/2+poly (I:C)] and cellular responses were compared with those produced by HVT-RD, HVT-1/2, 50µg poly (I:C), and vaccine diluent [sham-inoculated]. Frequency of various immunophenotypes in the spleen of 1-day-old meat-type chickens was evaluated by flow cytometry. Findings showed HVT-RD induced significantly higher frequencies of CD8+ and CD4+ T cells expressing activation molecules, CD28, CD44 or major histocompatibility complex class II (MHC-II), when compared to all other groups. All treatment groups induced significantly higher frequencies of T cell receptor (TCR) gamma delta (γδ) cells and activated macrophages (KUL01*MHC-II+) when compared to the sham-inoculated group. No poly (I:C) adjuvantation-mediated dose-sparing effects were observed. Furthermore, we show that in ovo vaccination with HVT-RD in meat-type chickens is a strong activator of CD4+ and CD8+ T cell subsets, macrophages, and TCR γδ cells, for which major effects were significantly superior to 50µg poly (I:C).

Funding Source: self-funded (revenue account)

Primary subject category for presentation: Immunology, Infectious Disease
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is a pathogen that heavily impacts the porcine industry through high rates of morbidity, mortality, and dramatic economic losses. Currently, PRRSV is only partially managed and controlled through vaccination strategies. Selenium (Se) is a micronutrient and feed additive used in swine that is essential to selenoprotein formation. Selenoproteins help protect against oxidative stress and have immunostimulating properties. These properties have led to investigations on how selenium supplementation can impact immune functions during viral infections. The purpose of this research is to investigate the antiviral properties of Selenium to determine its potential as a treatment during PRRSV infection. Different selenium-based compounds have been investigated; however, for this study, L-Selenomethionine was tested at three different concentrations: 0.3, 0.03, 0.003 ppm (parts per million). The highest concentration legally permitted in the swine industry is 0.3 ppm due to its potential toxicity. MA 104 cell line (African Green Monkey kidneys) was used for PRRSV infection, since it is the standard cell line for PRRSV studies. The compound was also used with PRRS viruses of different virulence and with a modified live virus vaccine strain. The results obtained showed a decreased viral load in cells treated with L-Selenomethionine in comparison to untreated cells in all tested viral strains. *In vitro* data indicate that L-Selenomethionine has the potential to be used as an antiviral for PRRSV infection, but additional experiments are needed to fully confirm its effectiveness, particularly in lung macrophages, which are the target of the virus, and in *in vivo* studies.

Funding sources:
Research Grant: PI Startup Funds
Student Support: NC State Veterinary Scholars Program

Category: Infectious Disease
FILTERED LUMINAL CONTENTS PROMOTE EPITHELIAL RESTITUTION ONLY IN CO-CULTURE WITH ENTERIC GLIA

**Madison Caldwell (DVM/PhD student)**
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NCSU CVM

Mucosal enteric glial cells (EGCs) promote epithelial restitution following injury to prevent devastating outcomes from intestinal barrier dysfunction. In mice, postnatal maturation and maintenance of mucosal EGCs have been shown to require gut colonization by luminal microbiota. Our lab previously found that poorly restituted neonatal intestinal tissue was rescued by application of juvenile mucosal homogenate during *ex vivo* recovery following surgical ischemia in a porcine model. *In vitro* porcine tissue studies have shown that epithelial monolayers co-cultured with EGCs enhances restitution following scratch wounding. We hypothesized that adult not neonatal luminal contents would further improve restitution in neonatal epithelial monolayers co-cultured with neonatal EGC. We compared the effect of sterile-filtered neonatal or adult luminal contents in the apical chamber on scratch wound restitution in IPEC-J2 monolayers in monoculture or co-culture with neonatal or adult EGC in a transwell system. Results of an ANOVA demonstrated an overall effect of EGC co-culture on IPEC-J2 restitution rates (biological replicate n=1, P=0.0028) and an interaction between EGC co-culture and treatment with luminal contents (P=0.3856). Compared to untreated controls, monocultured IPEC-J2 monolayers demonstrated decreased restitution when treated with neonatal luminal contents (P=0.037) and adult (P=0.023), while neonatal EGC co-culture accelerated restitution when treated with adult luminal contents (P=0.0024) and adult EGC co-culture accelerated restitution when treated with neonatal luminal contents (P=0.037). These data suggest that EGCs provide signals in response to luminal contents that promote repair. Future work aims to determine what is driving these differences in restitution by analyzing neonatal and adult luminal secretomes.

Funding sources: 5 T35 OD 11070-12
Subject category: Gastroenterology
CRISPR/CAS9 MEDIATED INACTIVATION OF ADENOMATOUS POLYPOSIS COLI (APC) IN COLONOIDS TO GENERATE A PORCINE MODEL OF COLORECTAL CANCER

Amber Carter¹, undergraduate
Cecilia Schaaf¹, Kathryn Polkoff¹, Breanna Sheahan¹,², Jatin Roper², Liara Gonzalez¹, Jorge Piedrahita¹
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Colorectal cancer (CRC) is the third leading cause of U.S. cancer-related deaths. While 80% of CRC tumors are initiated by mutations inactivating adenomatous polyposis coli (APC) tumor suppressor gene, the role of LGR5+ intestinal stem cells (ISCs) is unclear. To better understand LGR5+ ISCs in CRC pathogenesis, transgenic murine models are the current standard; however, mice do not fully recapitulate human pathophysiology. The pig is physiologically more similar to humans, but lack of reliable antibodies precluded LGR5+ ISC studies in this species. To address this, we developed a cell reporter pig expressing H2B-GFP under control of the LGR5 promoter (LGR5-H2B-GFP) that allows identification and purification of LGR5 stem cells, which can be grown in vitro as colonoids. We hypothesized to induce APC mutations in LGR5-H2B-GFP colonoids using CRISPR gene editing technology to mimic CRC and validate our model by observing tumor formation after engraftment into murine colons. LGR5-H2B-GFP colonoids were edited with either CRISPR ribonucleoprotein (RNP) or plasmid and compared through tracking of indels by decomposition. Nucleofection with RNP led to higher editing efficiency than with plasmid (11.25+/−0.05% vs. 0.7%). Further optimization of RNP protocol yielded the gene inactivation efficiency 42.4+/−0.008% (n=2). When grown in selective media, APC editing was further increased to 86.1%. To observe APCnull LGR5-H2B-GFP tumorigenicity, colonoids were transplanted via colonoscopy-guided submucosal injection into NSG mice (n=5). Resultant adenomas will be collected for histologic assessment of LGR5-H2B-GFP+ ISCs. This improved translational model of CRC will enhance our understanding of the role of LGR5+ ISCs in human CRC.

Funding: NIH R21OD019738, NIH NIGMS grant T34GM131947
Subject Category: Gastroenterology
Consideration: To be considered for oral or poster presentation
IMMUNE RESPONSE EVALUATION IN CLINICAL CASES OF TURKEYS AFFECTED WITH CLOSTRIDIAL DERMATITIS

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Clostridial dermatitis (CD), caused predominantly by Clostridium septicum anaerobic bacteria, is an economically important emerging disease of turkeys causing sudden deaths and necrotic dermatitis with edema and/or emphysema in the underlying subcutaneous tissues. Immune responses during CD infection are poorly understood. In the present study, C. septicum was isolated from turkeys clinically affected with CD and tissues (skin, muscle and spleen) were collected along with samples from clinically healthy birds to evaluate immune gene expression in local as well as systemic organs. The results showed that CD-affected turkeys had significantly higher levels of IL-1β, IFNγ and iNOS (inducible nitric oxide synthase) transcripts in the skin, muscle and spleen tissues compared to control birds. Affected birds also had a significantly elevated transcription of TLR21 gene in the skin and spleen tissues, suggesting a role for this toll-like receptor in the recognition of Clostridial pathogen. Additionally, the expression of IL-13 in the spleen and muscle and IL-4 in spleen tissues was significantly higher in the affected birds compared to controls. Furthermore, stimulation macrophages in-vitro with C. septicum led to a significant upregulation of IL-1β, IFNγ and iNOS gene expression compared to unstimulated controls. In summary, our results suggest a possible involvement of TLR21 in C. septicum recognition by cells of the avian immune system and that the CD infection can induce a robust inflammation accompanied by a T helper-2 (Th2) immune response in turkeys. The findings also indicate that C. septicum can effectively activate avian macrophages.

Funding source: US POULTRY and EGG ASSOCIATION

Primary subject category for presentation: Immunology
CHARACTERIZING THE INTRINSIC NERVOUS SYSTEM OF GALLBLADDERS FROM NORMAL DOGS AND DOGS WITH MUCOCELE FORMATION

Kaitlin V. DalyL veterinary student
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Gallbladder mucocele formation is one of the most common and deadly biliary diseases of dogs. The pathogenesis is poorly understood and treatment options are limited. Hallmark features are excessive secretion of abnormal mucus by the epithelium and poor gallbladder contractility. Secretion and contractility are both influenced by the intrinsic nervous system of the gallbladder. The purpose of this study was to develop methods to allow us to better characterize the intrinsic nervous system of the gallbladder in order to identify differences that may exist between healthy canine gallbladders and mucoceles. This included performing immunofluorescence on cryosections and whole mounts, and culturing the gallbladder glia. Cryosections were treated with antibodies specific for neuronal markers (Tubulin β3), glia (glial fibrillary acidic protein) and epithelium (EpCAM CD326). Our results show a subjective decrease in the amount of glia in mucoceles. Healthy canine gallbladders were dissected to separate the mucosa, muscularis and adventitia. To localize the ganglia, each layer was stained as a whole mount using immunofluorescence with antibodies specific for neuronal markers (Protein Gene Product 9.5, Tubulin β3). Ganglia were found primarily in the muscularis. The mucosa and muscularis were digested and cultured for glia, whose isolation was confirmed by immunofluorescence using antibodies specific for glia (glial fibrillary acidic protein, S100-β). As we move forward with this project, cultured glia will be used to study potential functional differences between mucoceles and controls. 3D imaging will be performed using iDISCO to more comprehensively characterize the intrinsic nervous system of the canine gallbladder.

Morris Animal Foundation Grant D17CA-068, George H. Hitchings New Investigator Award, CGS/IDEXX Veterinary Student Summer Scholar Award

Gastroenterology
DOGS OF CHERNOBYL: DEVELOPING A MODEL FOR HUMAN HEALTH EFFECTS ARISING FROM CHRONIC EXPOSURE TO RADIATION, HEAVY METALS, AND OTHER ENVIRONMENTAL TOXINS.

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Abstract:
In 1986, an explosion at the Chernobyl Nuclear Power Plant (NPP) released $10^{18}$ Bq of radioactive isotopes into the atmosphere, contaminating surrounding regions of Ukraine, Belarus, and Russia. Within 48 hours of the accident, the residents of Pripyat and several other towns within a 30 km radius of the NPP were evacuated. Authorities worried that animals left behind could spread radiation and sent in teams of “Liquidators” to euthanize agricultural livestock and abandoned pets. Some dogs managed to escape destruction. Today, a population of several hundred semi-domesticated animals live around the NPP. The region is still heavily contaminated by 137-Cs, 90-Sr, heavy/toxic metals, organics, and chemicals left from the accident and from decontamination efforts. The effects of these toxic exposures on the genetics of the resident canine population are still unclear. This study focuses on two populations of semi-feral dogs with varied exposures: one living around the NPP and another living ~13 km away in Chernobyl City. Through population genetic analyses, we hope to 1) identify local adaptation and differential expression between the NPP and Chernobyl City populations and 2) relate these genetic impacts to the chronic exposures and animal health. A growing dependance of nuclear energy as well as the subsequent Fukushima disaster highlight an urgent need to better understand how such exposures can adversely impact the genome and epigenome. Findings from this population of dogs can provide vital insights concerning identification of biomarkers of human exposure that can predict subsequent adverse health outcomes after future environmental disasters.

Funding Sources: Cancer Genome Fund, Genetics and Genomics Scholars Fellowship

Subject Category: Genetics
EVALUATION OF NEOPLASIA IN ZEBRA SPECIES IN THE WILD AND UNDER HUMAN CARE

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Oncology

There have not been many reports of evaluations of neoplasia in zebra species in the literature. There have been reports of equine sarcoilds, a result of bovine papillomavirus, in zebra in South Africa. A thorough review of zebra neoplasia in Kenya has not been done. Zebra in Kenya were reported to have sarcoilds present. These sarcoilds are most common in the legs, head, and lips and are often diagnosed through DNA samples of the affected fibroblast. This study collated data to not only to determine the occurrence of sarcoilds in zebra in Kenya, but to also to determine what are the different tumor types and which zebra species were most heavily affected by tumors in zebra under human care. Data was collated into an oncology database (Exotic Species Cancer Research Alliance, ESCRA). It was found that the most common zebra species reported in this data was the plains zebra (Equus quagga) with the most reported neoplasia as a sarcoild. The most common location for zebras with sarcoilds in Kenya was in the Lake Navisha area out of both wild zebra with a sarcoild was in a wildlife reserve or farm. For zebras under human care in a zoological institution, the most reported neoplasia were leiomyosarcomas and rhabdomyosarcomas. Additional research is underway to determine if there are additional cases of zebras under human care with neoplasia.
Tendons, specifically digital flexors, are common sites of trauma for performance animals and athletes. Translational research seeks to improve the efficacy of tendon healing via the manipulation of innate cellular components and their cytokinetic cross-talk with infiltrating immune modulatory cells. However, progress has been hindered by a lack of in vivo examination regarding the timing and presence of cytokines relevant to the tendon healing process. The aim of this study was to assess in vivo temporal expression of inflammatory cytokines during acute tendon injury using an equine model to enhance tendon targeted mesenchymal stem cell (MSC) therapy. For the study, novel BASi® 100 kDa ultrafiltration probes were inserted into surgically created superficial digital flexor tendon (SDFT) core lesions in six horses. Tendon ultrafiltrate was collected over 21 days; levels of IL-1β, IL-6, IL-8, IL-10, TNF-α, PGE2 and TGF-β isoforms were measured via multiplex immunoassay and mapped according to concentration and timing of detection. Descriptive statistical analysis was performed using SAS 9.4 and R software. Median concentrations of 2097 pg/mL of IL-1β, 1971 pg/mL of IL-6, and 110 pg/mL of IL-8 expression were recorded within the first 48 hours of collection. Going forward, MSCs will be stimulated with IL-1β and IL-6 in vitro; gene expression will be analyzed via RNA sequencing to measure changes in their secretome compared to unstimulated controls. This data will then be used to determine if further benefit can be conferred with inflammatory priming of MSCs for treatment of tendon injuries to improve current clinical therapy.

The Grayson-Jockey Club Research Foundation
Fund for Orthopedic Research in honor of Gus and Equine Athletes
The Barton & Marie-Claude White Equine Orthopedic Research Endowment

NIH: T35OD011070 (IE) IBRTP, North Carolina State University

Category: Immunology
PREVALENCE OF RADIOGRAPHIC OSTEOARTHRITIS AND ASSOCIATED CLINICAL SIGNS IN YOUNG DOGS

Masataka Enomoto (Staff)

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Abstract

No comprehensive, prospective studies of the prevalence of canine osteoarthritis (OA) throughout the skeleton have been performed and current estimates of OA prevalence pertain to older dogs despite the fact that OA in dogs is primarily driven by developmental joint disease. The aim of this study was to determine the prevalence of OA and associated clinical signs in young dogs.

Owners (n=320) of dogs aged 8 months to 4 years old from a single practice (NCSU CVM Primary Care), were contacted to participate (regardless of dog’s health status). Owners were contacted in random order within each of 4 age bands (8-18, 18.1-28, 28.1-38, 38.1-48months). Full clinical and orthopedic examinations were performed. Orthogonal radiographic projections of all joints and the spine were made under sedation. Owners completed OA questionnaires. Each joint was scored for radiographic OA severity on an 11-point scale by 2 investigators (ME, BDXL).

Owners of 123 dogs agreed to participate. Overall, 40.7% of dogs had radiographic appendicular joint OA (rOA) in at least one joint, and 33.3% of dogs had ‘clinical OA’ (cOA) as defined as overlap of radiographic OA and joint pain in the same joint. Owners of dogs with cOA observed signs of impairment in 44% of cases but only 11% of them were treated. Affected joints in descending order of frequency were elbow, hip, tarsus, and stifle. Prevalence of rOA was increased with age, and bodyweight. Radiographically visible OA is very common in young dogs, and approximately 82% of dogs with rOA had cOA.

Funding: Elanco Animal Health

Primary subject category: clinical medicine
MULTI-INSTITUTIONAL RETROSPECTIVE CASE-CONTROL STUDY EVALUATING CLINICAL OUTCOMES OF FOALS UNDERGOING SURGICAL CORRECTION OF STRANGULATING LESIONS OF THE SMALL INTESTINE: 2000-2020

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Retrospective case-control studies provide prognostic information for consideration when referring colic patients. Short-term survival rates of 50-80% have been reported in adult horses with small intestinal strangulating obstruction (SISO), while rates of 27-50% have been reported in foals, but age-dependent outcomes have not been compared directly. This retrospective case-control study examined differences in clinical outcomes between adult and foal SISO patients. Hospital records for surgical SISO cases were collected from North Carolina State, Colorado State, University of Pennsylvania, The Ohio State University, and University of California, Davis equine referral centers. Foals were ≤6-months-of-age, and adult controls were 2-20-years-of-age. Common adult lesions included strangulating lipomas (n=36), volvulus (n=25), and inguinal hernias (n=10). Common foal lesions included volvulus (n=22), intussusception (n=5), and mesenteric rents (n=3). Data revealed 25 of 41 (60.98%) foals and 75 of 105 (71.43%) adults were recovered from surgery. Of the foals recovered from surgery, 24 (96.0%) survived to hospital discharge. Of the adults recovered from surgery, 66 (88.0%) survived to hospital discharge. Of 16 non-surviving foals, 15 (93.75%) were euthanized intraoperatively and 1 (6.25%) was euthanized following recovery from anesthesia. Of 38 non-surviving adults, 28 (73.7%) were euthanized intraoperatively, and 10 were euthanized postoperatively. Survival analysis indicates foals are 3.273 times more likely to survive than adults (P=0.44, 95% CI 0.4667-37.28). The results of this study demonstrate no significant difference in outcome of surgically treated SISO foals compared with adults. The clinical application of such findings supports more optimism toward surgical treatment of foals with SISO.

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Category: Gastroenterology or Clinical Medicine
In naturally cycling female rodents, high levels of ovarian hormones during the proestrus phase of the estrous cycle decrease anxiety-like behavior compared to low levels during diestrus. However, the cellular mechanisms driving the anxiolytic effect of proestrus remain unknown. Activity of the basolateral amygdala (BLA), the brain’s emotional switchboard, is tightly regulated by GABAergic parvalbumin interneurons (PVIs). Previous studies indicate that PVIs express hormone receptors. We therefore hypothesized that ovarian hormones regulate BLA PVI activity to drive anxiety-like behavior across the estrous cycle. To test this, we performed whole-cell patch clamp electrophysiology on BLA PVI in PV-Cre reporter mice. We first validated Cre-recombination specificity by staining for PV and GAD67. We found that PVIs exhibit hyperpolarized resting membrane potential and reduced frequency of miniature excitatory postsynaptic currents in proestrus compared to diestrus, indicating decreased PVI excitation in proestrus. We then hypothesized that increasing BLA PVI activity would eliminate the anxiolytic effect of proestrus. To do so, we injected Cre-dependent excitatory DREADDs into the BLA of male and female PV-IRES-Cre mice and validated that CNO administration induced equivalent c-fos expression in BLA PVIs in males versus females. We activated BLA PVIs prior to behavioral testing. Chemogenetic activation of PVIs reversed the anxiolytic effects of proestrus while having no effect on males or diestrus females in the elevated plus maze or open field. Our data supports a model for decreased BLA PVI activity in proestrus females and further implicates a role for cycling ovarian hormones in affective behaviors.
RETROSPECTIVE STUDY OF FISH CANCER PREVALENCE AND THERAPIES

Emma Ferraro, Leigh Duke, Tara Harrison

North Carolina State University College of Veterinary Medicine, Exotic Species Cancer Research Alliance

This study evaluated fish cancer through medical records from zoos, aquariums and exotic animal veterinarians. The parameters evaluated included geographic location, habitat type, signalment, location of cancer, type of cancer, survival time, and treatments provided for each patient. This data was entered into the Exotic Species Cancer Research Alliance (ESCRA) database, then the resulting statistics were compiled and analyzed. Out of 511 cases, sex was known from 28.4% of patients, and most were female (14.5%). A majority of the submitted animals were from a zoological park or aquarium (57.3%), followed by private ownership (9.8%). The most common species reported was koi (spp. Cyprinus rubrofuscus) (25.0%). The most commonly specified location of primary tumors was the neck (7.0%), with soft tissue being the most commonly affected body system (22.1%). Spindle cell sarcoma was the most common primary histopathology tumor diagnosis (12.7%). Most of the reported cancers were malignant (65.2%). Only five cases reported any form of treatment for the tumors, with surgery being the only therapeutic method used. Four of these treated patients had their cancer completely excised and one animal had an incomplete excision of their cancer. None of these animals were reported to have any complications or had died as a result of their surgical procedures. This data suggests that there is a deficit in documented clinical therapy of cancer in fish species. However, despite this limited data, surgical excision of cancer in fish may be beneficial for clinicians to strongly consider or further investigate when feasible for the patient and client.

Research Grant and student support: EF, NC State Veterinary Scholars Program and the Boehringer Ingleheim Veterinary Scholars Program
INVESTIGATION OF PROTOCADHERIN-8 INTERACTING PARTNERS IN INTESTINAL EPITHELIAL STEM CELLS

Abi Fetzer (Undergraduate)

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Abstract: The intestinal epithelium has a high rate of continuous cell turnover driven by intestinal epithelial stem cells (ISCs). Stem cell self-renewal is dependent on the ability of ISCs to interact with their niche. Protocadherin-8 (Pcdh8) is a transmembrane protein that belongs to a subgroup of Cadherins involved in cell-cell adhesion. Pcdh8 was found to be significantly enriched in ISCs, although the role of Pcdh8 in these ISCs has not been described. The goal of this work was to identify potential interacting partners of Pcdh8 to better understand its function in ISCs. To answer this question, we performed co-immunoprecipitation (co-IP) followed by mass spectrometry and identified epithelial protein lost in neoplasm (EPLIN), plakoglobin (γ-catenin), lysine-specific demethylase 5C (KDM5C), and nuclear receptor coactivator 1 (NCOA1) as potential Pcdh8 interactors. To validate these interactions, we used western blot analysis of co-IP samples. We then performed immunofluorescence and proximity ligation assays (PLAs) to test whether these candidates colocalize with Pcdh8. Through PLA and western blot analysis, we were able to confirm that Pcdh8 interacts with EPLIN and plakoglobin in ISCs. We are now pursuing these investigations and exploring the impact of these interactions on cell-cell adhesion and chromatin regulation in ISCs.

Primary Subject Category: Cell Biology
EFFECTS OF CHICKEN LACTOBACILLUS ISOLATES ON MACROPHAGE FUNCTION AND ABILITY TO INHIBIT CLOSTRIDIUM PERFRINGENS BACTERIA

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Abstract: Necrotic enteritis (NE) in chickens is an economically important disease caused by Clostridium perfringens bacteria. In the current era of ‘no-antibiotic-ever’ farming, NE incidences are on the rise and Lactobacillus-based probiotics seem to offer a promising non-antibiotic alternative. Here, we used four Lactobacillus species, L. crispatus (C25), L. animalis (P38), L. acidophilus (P42) and L. reuteri (P43) to evaluate their in-vitro effects of macrophage function and their ability to inhibit C. perfringens growth. The results showed that while macrophage stimulation with P42 strain induced a significant transcriptional upregulation of pro-inflammatory cytokines (IL-1β, IL-6, IFNγ), the C25, P38 and P43 stimulation led to a significantly decreased expression of IL-1β and iNOS (inducible nitric-oxide synthase) genes compared to unstimulated control. Additionally, P38 and P42 strains induced a significantly increased transcription of TLR2 gene, suggesting a role for this toll-like-receptor in macrophage recognition of lactobacilli. Furthermore, treatment of macrophages with Lactobacillus cell-free supernatants (CFS) showed that while P42 and P43 could significantly increase the expression of IL-1β, IL-6, IFNγ genes, C25, P38, P42 and P43-derived CFS-treatment led to a significantly reduced transcription of CD40 and CD80 costimulatory genes coupled with significantly elevated IL-10 gene expression. Using agar-spot, well diffusion and co-culture assays, all the Lactobacillus species/strains showed varying degree of C. perfringens growth inhibition with P38 and P42 producing marked effects. Taken together, the present work shows that certain lactobacilli can possess superior anti-C. perfringens activity and that the activation or modulation of macrophage function seems to depend on the Lactobacillus species/strain.

Funding Sources: PI Start-up
Primary Subject Category: Immunology
Novel severe acute respiratory syndrome virus 2 (SARS-CoV-2) resulted in the coronavirus disease 2019 (COVID-19) pandemic. Although not definitive, COVID-19 is thought to have spilled over to humans via an intermediate host, with bats and pangolins serving as possible initial hosts and coronavirus evolving through another intermediate (yet undetermined) species prior to establishment in humans. Companion animals (dogs and cats) are not considered to be significant reservoirs of SARS-CoV-2 at this time but understanding the movement of SARS-CoV-2 through animals during this pandemic may help build a stronger understanding of future pandemics or variants of SARS-CoV-2. Additionally, veterinarians need to understand the prevalence of SARS-CoV-2 when examining dogs and cats with respiratory illness during peak transmission times so as to treat and quarantine patients appropriately. We aimed to determine the prevalence of SARS-CoV-2 in a population of dogs and cats living with humans positive for the virus via PCR on day 0+ and serology (IgM and IgG) on day 28+. Oral and rectal swabs were collected from dogs and cats for PCR, and blood samples for serology. Test results were validated using negative controls and serial testing of positive samples.

Subject Category: Infectious Disease

Impacting veterinary standard of care
LYMPHOID MUCOSAL IMMUNE RESPONSES AGAINST CLOSTRIDIUM PERFRINGENS AFFECTING BROILER CHICKENS

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*Clostridium perfringens* type A bacteria cause necrotic enteritis (NE), an economically important disease, in chickens. Although the NE pathogenesis is well-studied, the immune responses against *C. perfringens* are poorly understood. In the present study, we used an experimental NE model to characterize immune responses against *C. perfringens* isolates that varied in their pathogenicity. Broiler chickens were challenged with CP5 (avirulent), CP1 (virulent) and CP26 (very virulent) along with controls to evaluate expression of immune genes (IL-1β, IL-6, IFNγ and IL-10) in the cecal tonsils (CT), bursa and harderian gland (HG) tissues as well as measure antibody responses. Additionally, macrophages were stimulated in-vitro with *C. perfringens* bacilli to measure expression of IFNγ gene and nitric oxide production. Results indicated that while CP26 induced an upregulated expression of IFNγ, IL-6, and IL-1β genes in CT and increased IL-10 expression in the bursa, the CP5 infection led to a decreased IL-1β gene expression in HG tissues, indicating a spatially regulated inflammatory response by *C. perfringens* that vary in their level of virulence. The humoral response evaluation showed that CP26 could induce a robust IgM antibody response compared to controls. Furthermore, macrophage stimulation with *C. perfringens* in-vitro also led to an increased IFNγ transcription as well as nitric oxide production. In summary, the present study shows that *C. perfringens* can induce an inflammatory response in certain lymphoid tissues associated with an increased antibody response in-vivo as well as macrophage activation in-vitro, and that these responses depend on the virulence nature of this pathogen.

Subject: Immunology
Pet relinquishment to animal shelters has not been comprehensively studied in over 20 years. Relinquishment reasons should be reevaluated in relation to demographic and geographic factors across the United States to appropriately advise community and nationwide intervention strategies. To update this information, an online survey was developed to capture reasons for dog and cat relinquishment to shelters across six geographical regions (Northeast, Southeast, Midwest, Southwest, Rocky Mountain, and Pacific) in the United States. This survey asks shelters to record the reasons owners listed for relinquishing their pets in the years 2019 and 2020. Categories for relinquishment were based on previous studies: animal behavior, socioeconomic and personal factors, and owner or animal health. In addition to relinquishment data, questions to determine the demographic and socioeconomic status of the communities served by each shelter, current intervention strategies being practiced by shelters, and changes in operation resulting from the COVID-19 pandemic in the year 2020 were asked. When possible, information provided by shelters will be cross referenced with the most current US census data. After piloting the survey among 10 shelter professionals, the survey is currently gathering data via advertisement by large nonprofit organizations and social media to shelter professionals across the country. The resulting data is expected in the Summer of 2022 for statistical analysis.

Goss: NIH-T-35 Interdisciplinary Biomedical Research Training Program (IBRTP). Gremling: Boehringer Ingelheim Veterinary Scholars Program (VSP).

Clinical Medicine/Other
SEROVAR-LEVEL DETECTION OF BACTERIAL FOODBORNE PATHOGENS USING LONG-READ AMPILICON SEQUENCING

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Funding Source: USDA NIFA Grant 2019-67021-29927
Subject Category: Infectious Disease

Abstract:

Foodborne illnesses are the cause of many hospitalizations and deaths annually across the globe and are a significant threat to public health and safety. *E. coli* and *Salmonella* species both contribute to many of these hospitalizations and are an increasing health risk as global trends show that the health issues caused by these pathogens are increasing every year. Laboratories which study these pathogens have been collecting extensive high-throughput sequencing data over the last decade across many different serovars of *E. coli* and *Salmonella*. The GenomeTrakr database is a global project focused on consolidating sequencing data on hundreds of thousands of pathogenic bacterial samples. In combination with long-read amplicon sequencing, this project aims to use the data available in the GenomeTrakr database to construct ribosomal marker-gene databases which can be parsed for the identification of pathogenic bacterial species. The primary goal is to develop a fast and accurate identification method which will allow for serovar-level identification of target pathogenic bacteria from food samples using long-read amplicon sequencing data. We have developed and validated a computational pipeline which we have shown is able to make accurate serovar predictions for both *Salmonella* and *E. coli* from 16S ribosomal amplicon sequences, currently implemented in the form of an R software package. In the past, serovar assignment has been done through sequencing of various established marker genes, and this is the first demonstration of serovar prediction through a universal multi-copy marker gene approach.
AN UPDATED DESCRIPTION OF BACTERIAL PNEUMONIA IN HORSES AND FACTORS ASSOCIATED WITH NON-SURVIVAL

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Bacterial pneumonia causes significant morbidity and mortality in horses. Available research describing common bacteria, antimicrobial susceptibility, and factors associated with survival are outdated or focus on specific horse or bacterial populations. The objective of this paper was to describe the clinical presentation and bacterial isolates of horses with bacterial pneumonia and identify factors associated with non-survival. 113 horses >2 years old with bacterial pneumonia were included in this retrospective case series. Data regarding history, physical examination, clinicopathologic features, bacterial culture and sensitivity, treatment, and outcome were collected.

*Streptococcus equi* subspecies *zooepidemicus* was the most commonly isolated bacteria (50%), followed by *Klebsiella* spp. (19.7%), other *Streptococcus* species (17.7%), *Escherichia coli* (16.6%), and *Bacillus* spp. (14.5%). *Streptococcus equi* ssp *zooepidemicus* isolates were highly susceptible (95.1%-100%) to all antibiotics tested aside from trimethoprim sulfa (66.7%). *Escherichia coli* and *Klebsiella* isolates showed reduced susceptibility to gentamicin (70.6%, 66.7%), enrofloxacin (87.5%, 50%), and sulfa drugs (47.1%, 55.6%). Survival to discharge was 71.1%. Tachycardia (OR 23.12, CI 95% 2.96-587.73) and elevated creatinine (OR 13.30, CI 95% 2.22-213.03) increased the risk of non-survival. Increasing albumin (OR 0.04, CI 95% 0.00-0.30) and lymphocyte count (OR 0.20, CI 95% 0.04-0.63) were protective against non-survival.

Although bacterial isolates were similar to historical studies, antibiotic susceptibility rates for *Escherichia coli* and *Klebsiella* spp were lower than previously reported. Changing antimicrobial susceptibility patterns may influence empiric treatment decisions. Increased risk of non-survival associated with tachycardia and azotemia is consistent with previous studies in horses and can be utilized in initial case assessment.

Subject: Clinical Medicine
INDICATIONS OF ENTERIC GLIAL IL-1 SIGNALING IN EQUINE POSTOPERATIVE ILEUS

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Primary Subject Category: Gastroenterology
Poster Presentation

Abstract:

Postoperative ileus (POI), a “delay in the return of normal gastrointestinal motility following surgery,” is a devastating complication of equine colic surgery, reporting mortality rates as high as 85.7%. POI is incited neurogenically and amplified by inflammation within the intestinal wall, drawing inflammatory cells and their anti-motile products. However, the mechanisms linking these phases have not been fully defined. Enteric glial cells (EGC), through sensing the intestinal environment and coordinating responses to injury, could be significant in this link. Our long-term goal is to determine the mechanism through which stimulated EGC directly increase intestinal barrier permeability and propagation of inflammation in POI.

In a murine POI model, IL-1 receptor blockade was capable of preventing POI formation. Additionally, inflammatory cytokines produced by IL-1β-stimulated EGC are capable of altering intestinal barrier function. Therefore, we hypothesized that mucosal IL-1β levels and EGC IL-1β sensitivity would be increased in horses that develop POI. Small intestinal resection site margins were evaluated by IL-1β ELISA and immunofluorescence of submucosal EGC IL-1 receptor. Horses with indications of POI were defined as those who refluxed greater than 2 liters postoperatively.

Preliminary results of mucosal IL-1β levels indicate a trend towards increased levels within the proximal resection site of horses that develop POI compared to those who do not (p=0.1439). In addition, immunofluorescent quantification indicates increased submucosal EGC IL-1 receptor expression in horses that develop POI (p=0.0441). Further studies will provide insight into the role of EGC IL-1 signaling in equine POI pathophysiology and highlight a potential therapeutic target.

Source of funding: Morris Animal Foundation
THE INHIBITORY EFFECTS OF ROTENONE ON INTESTINAL GROWTH IN XENOPUS LAEVIS

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Rotenone, a commonly used pesticide, is a known mitochondrial respiration inhibitor. To investigate the effects of this compound on the development of living organisms, we treated Xenopus laevis embryos with rotenone (150nM) during organogenesis and scored for developmental defects at tadpole stages. Interestingly, we found that the intestines of the rotenone-treated embryos were significantly shorter than controls, suggesting that the process of gut elongation is highly susceptible to decreased mitochondrial respiration. Additionally, immunohistochemical (IHC) staining revealed that the intestines of treated embryos had a high number of rounded cells with microtubule asters, reminiscent of cells in mitosis, as opposed to the columnar cells present in controls. Furthermore, rotenone-treated intestines showed higher expression of phosphohistone-H3, specifically in the round cells, suggesting they are arrested in mitosis. Later, these undivided cells die by apoptosis and slough off into the lumen of the gut. Because rotenone-treated intestines are shorter, this led us to hypothesize that rotenone cells fail to divide into daughter cells, which then fail to intercalate back into the epithelium to contribute to tissue lengthening. Altogether, our results reveal the importance of mitochondrial respiration during intestine elongation.

Primary Subject Category: Cell Biology

Funding Source: R01 HD089243, National Institutes of Child Health and Human Development
ASSESSING EARLY POSTNATAL DEVELOPMENT OF THE ENTERIC GLIAL NETWORK

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The enteric glial network is instrumental in intestinal barrier maintenance and repair, but is immature at birth. At birth, enteric glia are restricted to the submucosal and myenteric plexuses, and are driven to populate the lamina propria by microbial colonization and changes in microbial populations at weaning. Our lab uses a comparative pig model to describe and quantify early postnatal development of the enteric glial network. We hypothesized the density and distribution of glial cell subtypes would change within the early postnatal period, illustrating early postnatal development and maturation of the enteric glial network. Using the iDISCO three-dimensional imaging technique, the glial network in 1-, 7-, 14-, and 21-day-old pigs was analyzed by adapting an algorithm to quantify the volume, maximum and mean intensities of glial markers of GFAP, Sox10, and S100β within manually isolated villi. In the lamina propria, GFAP density by volume decreases (P=0.0228) while S100β increases (P=0.0019) from 1- to 21- days. Further research will compare the effects of high and low concentration oligosaccharide diets with the rationale that supplementing prebiotics will drive the establishment of key populations, creating a microbiome more similar to an adult pig. This modified microbiome may direct glial cells to migrate into the villi sooner after birth, leading to enhanced ability of neonates to repair following ischemic injury. This type of diet adaptation may be a candidate for later use as possible prophylactics in the swine industry and have potential application in human infant formula.

Subject Category: Gastroenterology
CLONING AND EXPRESSION OF CLOSTRIDIUM SEPTICUM NON-TOXIC ALPHA-TOXIN DOMAINS AS RECOMBINANT PROTEINS FOR IMMUNIZATION AGAINST CLOSTRIDIAL DERMATITIS IN TURKEYS

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Clostridial dermatitis (CD) affects commercial turkeys and chickens causing severe economic losses to the poultry industry in the United States and worldwide. *Clostridium septicum* alpha-toxin (CSA), a pore-forming necrotizing cytolysin, has been implicated as the key virulence factor CD pathogenesis and antibodies against CSA have been shown to play an important role in protective immunity. Despite the importance of CSA in CD immunity, no effective vaccine is currently available against CD in poultry. The overall objective of the present study is to identify two non-toxic alpha-toxin domains, ntATX-D1 and ntATX-D2, and clone and express them as recombinant proteins for use as subunit vaccines against CD in turkeys. While the ntATX-D1 sequence (912bp size) for cloning was designed such that the protein product lacks the proteolytic cleavage site critical for toxin’s activity, the ntATX-D2 segment (531bp size) was devoid of signal peptide sequence and the region corresponding to the cytolytic, pore-forming domain of CSA. The ntATX-D1 domain was cloned into pET14b expression vector using *E. coli* DH5α (shuttle host) and BL2-DE3 (expression host) and purified as a histidine-tagged recombinant protein. The expression and purification of recombinant ntATX-D1 protein from *E. coli* was confirmed by SDS-PAGE and Western blot analysis. Similarly, the cloning, expression and purification of ntATX-D2 is presently underway. Further studies will use the recombinant ntATX-D1 and ntATXD2 proteins for immunizing turkeys against CD.

Funding source: US POULTRY and EGG ASSOCIATION

Primary subject category for presentation: Infectious disease
OPTIMIZATION OF A QUBIT FLUOROMETER ASSAY FOR THE QUANTIFICATION OF CFDNA IN EQUINE PLASMA

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Colic is the leading cause of death in horses. Early intervention for severe colic can decrease patient morbidity and mortality, therefore novel biomarkers to assist with colic diagnosis and prognosis are needed. Cell free DNA (cfDNA), released during cellular apoptosis, necrosis and NETosis, has been investigated as a biomarker for gastrointestinal disease and inflammation in other species. Previous experiments in our lab using the tabletop Qubit fluorometer have shown that direct and extracted plasma cfDNA are increased in horses with colic, compared to healthy horses. However, we also detected inaccuracies with direct measurement of cfDNA due to suspected matrix effects of equine plasma. The purpose of this study is to use banked equine plasma samples to optimize the Qubit™ dsDNA HS (High Sensitivity) Assay Kit (ThermoFisher) for accurate measurement of cfDNA in equine plasma. We hypothesized that dilution of equine plasma would increase the accuracy of Qubit-measured cfDNA in equine plasma. Linearity of dilution experiments with the Qubit and NanoDrop One were significantly correlated and showed good linearity. However, treatment with DNase I, and spike and recovery experiments, showed matrix effects persist up to 1:16 dilution. These findings were consistent with standard addition plasma optimization experiments, which showed that 1:32 and 1:64 plasma dilution is needed to minimize matrix effects. Investigation of clinical applications of the direct measurement of cfDNA as a biomarker in dilute equine plasma is ongoing.

Research Grant: The Foundation for the Horse Graduate Student Research Grant

Student Support: Veterinary Scholars Program

Primary Subject Category: Clinical Medicine
Clostridioides difficile toxins increase FXR gene expression in colonic cells

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Primary Category - Gastroenterology/Infectious Disease/Cell Biology

Clostridioides difficile is a gut microbe responsible for C. difficile infection (CDI), an intestinal disease with increased morbidity rates due to lowered colonization resistance resulting from prior antibiotic use. Antibiotic treatment for CDI exists but isn’t always effective as recurrent infection occurs in 30% of cases; understanding the mechanisms by which C. difficile promotes infection will allow for improved treatment. Altering the bile acid synthesis pathway is one such option, as primary bile acids are shown to support C. difficile proliferation while secondary bile acids inhibit its growth. Farnesoid X-receptor (FXR) is a host transcription factor that suppresses the bile acid synthesis pathway. C. difficile produces two toxins, Tcd-A and Tcd-B, that alter cellular pathways in host cells. Investigating the effect these toxins have on FXR expression in human colonic cells provides information on how CDI influences the host, which can be used to design more effective treatments that lower instances of recurrent infection. Using qRT-PCR, FXR gene expression was assessed in both Caco-2 and HCT116 cells treated with and without toxins. Results showed C. difficile toxins increase expression of FXR. Assumptions can be made that C. difficile toxins activate FXR to suppress secondary bile acid production that normally impedes C. difficile growth, allowing microbe proliferation; therefore, treatments that inhibit this process can be designed. Future research involves determining the specific toxin responsible for altering FXR and verifying if this same response occurs in a CDI mouse model.
INVESTIGATING DIAGNOSTIC UTILITY OF AGAROSE CELL BLOCKS PREPARED FROM HEPATIC AND SPLENIC ASPIRATES

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Cytologic evaluation of fine needle aspirates is an inexpensive and minimally invasive diagnostic technique, but advanced testing (e.g immunocytochemistry) on these samples is limited by small and unevenly distributed samples and lack of control materials. Cell blocks are prepared by embedding aspirates into a solid matrix, which can then be routinely fixed and processed in the histopathology lab. The aim of this study was to determine whether cell blocks of adequate cellularity can be prepared from hepatic and splenic aspirates. Aspirates from canine cadavers were expelled directly into warmed liquid agarose. Samples were embedded into agarose, formalin-fixed, paraffin-embedded, and sectioned for H&E and immunohistochemistry (IHC). Cell blocks prepared from fresh hepatic and splenic aspirates yielded high cellularity specimens with excellent cellular morphology. IHC for cytokeratin, vimentin, CD3, Pax5, and Iba-1 yielded expected results with minimal background. We conclude that agarose cell blocks can be prepared from aspirates of solid tissues. These findings expand the potential of utility of cell blocks beyond academic veterinary institutions.

Research Grant: NC State faculty startup funds

Student Support: NC State Veterinary Scholars Program and the Boehringer Ingelheim Veterinary Scholars Program

Other, Pathology
CHARGE syndrome phenotype penetrance is related to location-specific mutations in CHD7

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CHARGE syndrome is a rare congenital disease initially characterized by coloboma of the eye, heart defects, atresia of choanae, retardation of growth/development, genitourinary defects, and ear anomalies. Currently, CHARGE syndrome is diagnosed with expanded criteria including behavioral deficits and intellectual disability, but all of these symptoms are expressed on a spectrum and no two patients present identically. The majority of CHARGE cases are caused by loss of function, de novo mutations in a major transcriptional regulator, chromodomain-helicase-DNA-binding-protein 7 (CHD7). CHD7 is indispensable in specific neurodevelopmental processes and is required for proper neural crest cell development. While the link between CHD7 mutations and CHARGE syndrome is well established, whether the location of the mutation determines the set of CHARGE phenotypes that are expressed is unclear. Preliminary data from our lab using a larval zebrafish CHARGE syndrome model suggests that mutation location may play a role in phenotype penetrance and severity. Chd7 mutants with mutations targeting different exon locations display differences in morphological phenotype frequency and sensorimotor behaviors. To determine if a similar pattern is found in human CHARGE cases, we analyzed a data set of 118 patients from Legendre et al. (2017). Using the provided mutation locations and phenotype data for the patients, we identified protein domains within CHD7 related to a higher penetrance of phenotypes, notably the ATP helicase domains, DEXDc, HELICc, and histone interacting domain, SANT. These findings update our understanding of how mutations in CHD7 lead to disease pathogenesis and provide insight into CHARGE syndrome phenotype presentation.

Topics: Genetics, Neuroscience
Funding: NIH R21-NS120079-01A1; The Charge Syndrome Foundation
Fungal keratitis (FK) is a sight-threatening infection of the cornea that is increasing in prevalence worldwide. Correct antifungal selection and treatment are essential for the best visual outcome. There is also growing resistance to the drugs commonly used as first-line FK treatments. Therefore, novel, broad-spectrum antifungal drugs that can be used topically as well as models that reliably assess the efficacy of the drug and replicate the complexities of corneal disease are needed. This study was conducted to determine if in vitro MIC testing correlates with efficacy of Amphotericin B (AMB), Natamycin (NAT), Voriconazole (VOR), and Luliconazole (LUL) to inhibit fungal (Aspergillus and Fusarium) growth in the cornea, using an ex vivo porcine cornea model. All concentrations of VOR and the MIC and high concentration of NAT inhibited growth of Fusarium keratoplasticum, while the low concentration of NAT and all concentrations of AMB allowed for similar growth to the control. Comparatively, Fusarium falciforme incubated in the low concentration of VOR and the MIC and high concentration of NAT showed less inhibition of growth relative to F. keratoplasticum. Aspergillus flavus showed minimal growth when treated with all concentrations of NAT and AMB and variable results when treated with VOR. The MIC and high concentrations of all drugs inhibited growth of Aspergillus fumigatus, while the low dose of NAT allowed for slight growth and the low dose of AMB allowed growth similar to the control. We conclude the concentrations of AMB, NAT, and VOR must be higher than the MIC in the cornea to inhibit growth of F. keratoplasticum, F. faciforme, and A. flavus respectively, and all other drug/isolate combinations correlate to in vitro testing.
DECREASED EXPRESSION OF CYSTIC FIBROSIS TRANSMEMBRANE REGULATORY CHANNEL (CFTR) PROTEIN EXPRESSION IN GALLBLADDER EPITHELIUM OF DOGS WITH MUCOCELE FORMATION

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Mucocoele formation occurs when the gallbladder epithelium secretes excessive abnormal mucus often leading to obstruction or rupture. It is among the most common and deadliest causes of gallbladder disease in dogs and its cause is unknown. Hydration of gallbladder mucus is mediated by the cystic fibrosis transmembrane regulatory channel (CFTR) via secretion of chloride and bicarbonate. Prior studies showed that CFTR knockout laboratory animals (piglets and ferrets) develop gallbladders similar to canine mucocoeles. Accordingly, the objective of our study was to examine the expression of CFTR in gallbladder epithelium of dogs with mucocoele formation. We hypothesized that mucocoele formation is associated with decreased epithelial expression of CFTR. Protein was extracted from the gallbladder mucosa of dogs with and without mucocoele formation (8 each) and used in Western blotting to detect CFTR and E-Cadherin (a tight junction protein known to be present in gallbladder epithelium, used as an epithelial housekeeper to control for epithelium among samples). Canine kidney protein extracts were used as a positive control. Densitometry results demonstrate significantly decreased expression of CFTR relative to E-Cadherin in gallbladder mucocoele samples compared to normal gallbladders (P<0.01, Mann-Whitney rank sum). These findings support our hypothesis that decreased CFTR in gallbladder mucocoele epithelium may be a primary or contributory cause of mucocoele formation. Further study will evaluate mechanisms leading to decreased CFTR expression. These findings will contribute to our understanding of CFTR’s role in gallbladder epithelial secretion in dogs with mucocoeles and provide a possible treatment target for this deadly disease.

Morris Animal Foundation D17CA-068
Veterinary Scholars Program at North Carolina State University’s College of Veterinary Medicine

Gastroenterology
Preference: either oral or poster presentation
EVALUATION OF INTESTINAL ALKALINE PHOSPHATASE AS A NOVEL THERAPEUTIC FOR EQUINE INTESTINAL EPITHELIAL INJURY

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Gastrointestinal (GI) disease remains a leading cause of morbidity and mortality in horses. Therapeutic options remain limited, and it is necessary to investigate novel or alternative treatments to improve patient outcome. In recent literature, intestinal alkaline phosphatase (iALP) improved restoration of barrier function in murine models of GI disease. We hypothesized that treatment of equine intestinal epithelial cells (IESCs) with iALP would improve epithelial repair following injury. Equine jejunum (n=3) was collected following euthanasia. Intestinal epithelial stem cells were isolated, plated, and supplemented with growth factors. Enteroids were dissociated and replated for a 100% confluent monolayer. Monolayers were scratched and treated with media (control) or iALP at 1 unit/mL (low) or 10 units/mL (high). Scratch margins were monitored every 6 hours for cell migration and wound closure. Closure-percentage was calculated and compared between groups (p<0.05 considered significant). Wound closure occurred by 18-24 hours in all groups. Six hours post-injury, controls had increased closure-percentage (68.8%) compared to both low (57.4%, p<0.0001) and high dose iALP (59.8%, p=0.0003). At twelve hours, control monolayers closed 92.2% compared to low (90.1%) and high (86.7%) iALP treatment. There was no difference between low iALP and control, however high iALP closure was significantly decreased (p=0.01) compared to control. Preliminary results suggest that in vitro treatment of equine IESCs with iALP does not improve epithelial wound closure following scratch injury. Additional studies are needed to investigate effects on epithelial barrier function, dose-response, and alternative models of injury.

Funding Source: American College of Veterinary Emergency & Critical Care

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Subject Category: Gastroenterology
ESOPHAGEAL T-CELL INFILTRATION IN A PIG MODEL FOR EOSINOPHILIC ESOPHAGITIS (EOE)

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Food allergies are a serious concern costing the US $25B annually in children alone. A chronic form of food allergies known as eosinophilic esophagitis (EoE) is growing in prevalence and can impact people of all ages. EoE can lead to a child’s failure to thrive or esophageal fibrosis in adults. On top, there are currently no prevention or FDA-approved treatments for EoE. To address this health problem, the Käser lab works towards the establishment of the pig as a relevant biomedical animal model for EoE: long-term goals are i) to promote the development of treatment and prevention strategies, and ii) to improve the understanding of the underlying immune mechanisms of this T-cell driven disease. This project shall answer the question: Do food allergic pigs have more esophageal T cells? To create these food allergic pigs, pigs were sensitized and challenged with the food allergen hen egg white protein. Post challenge, control and food allergic pigs were sacrificed to collect their esophagi for fluorescent immunohistochemistry: Frozen tissue sections were stained for i) CLDN4 to visualize the different esophageal tissue layers, ii) DAPI to show cell nuclei and iii) the T-cell marker CD3. Stained sections were imaged using fluorescent microscopy. Currently ongoing are the quantification of the area and the infiltrated T cells of the esophageal lamina propria and epithelium. Preliminary results indicate that food allergic pigs have more esophageal T cells. If the full quantification confirms these preliminary results, it would indicate that T cells may play an active role in the local immune response in EoE. Future studies will then investigate if and how these esophageal T cells drive EoE in pigs.

Research Grant: NIH R21 (grant # 1R21AI149098-01)
Student Support: JO T35OD011070
THE CULTURED SWINE: THE CELLULAR RESPONSE OF PRIMARY PORCINE OVIDUCT EPITHELIAL CELLS TO CHLAMYDIA

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Chlamydia trachomatis is the most common sexually transmitted bacterial infection with sequelae including salpingitis, ectopic pregnancy, and infertility. Its prevalence, severe disease outcomes, and mixed antibiotic treatment results call for a vaccine; however, despite numerous efforts, none have made it to market. To improve the translatability of pre-clinical results, our overall goal is to develop a vaccine in a biologically-relevant large animal model – swine. A lack of translatability of mouse models could be the different mechanisms by which the most important immune molecule, the cytokine interferon-γ (IFN-γ), acts in mouse models versus humans – murine IFN-γ acts through iNOS pathways, and human IFN-γ through IDO. To further solidify the similarity between the porcine and human immune response to C. trachomatis, the specific goal of this project is to determine if porcine IFN-γ also suppresses chlamydial growth through the activation of IDO. To this end, primary porcine oviduct epithelial cells (pOEC) were isolated and infected with C. trachomatis in the presence of increasing amounts of IFN-γ. Our data show that IFN-γ strongly reduced chlamydial propagation in pOECs. Next, we will perform these infection studies in the absence or presence of an IDO inhibitor. Our hypothesis is that this inhibitor can largely prevent the effect of IFN-γ. If true, this would demonstrate that porcine IFN-γ, as in its human counterpart, acts through the activation of IDO. In turn, this similarity between human and porcine IFN-γ would further solidify the use of swine as a biomedical animal model for Chlamydia research and vaccine development.

Funding Sources: NIH NIAID 1R01AI162709-01
Primary Subject Category: Immunology
Intervertebral disc disease (IVDD) is the most common spinal cord disease in dogs. Little information is available regarding the unique clinical presentation of nerve root signature (NRS) due to IVDD. The aim of the study was to detail the clinical and magnetic resonance imaging (MRI) findings in dogs displaying NRS associated with cervical IVDD. Medical records from 2010 to 2020 were retrospectively reviewed. 47 client-owned dogs presenting with thoracic limb lameness not attributed to an orthopedic cause and MRI confirmed IVDD were included. Imaging studies were evaluated to characterize location and severity of neural tissue compression. The majority (77%) of dogs were >7 years old. The dachshund (n=10) was the most common breed, and there was a relatively even distribution of small (<10kg) (55%) and large breed (45%) dogs. Disc material was significantly more likely to be lateralized (p=0.0005) and involve C5-T2 discs (p=0.0009), however 42% cases involved C2-5 discs. The most commonly affected site was C6-7 (p=0.001), which was significantly more prevalent compared to historical canine IVDD populations. Severe nerve root compression was not found in all dogs, with some displaying absent compression entirely. This study confirmed that NRS is a clinical manifestation associated with lateralized IVDD often affecting the cervical intumescence, however it can be seen with disease anywhere along the cervical spine. The high prevalence of C6-7 intervertebral disc involvement suggests there may be unique anatomic factors that contribute to development of NRS at this site. NRS may be more common in older dogs.

Primary Subject Category: Clinical Medicine
Pogona vitticeps or bearded dragons have become common pets among the public throughout recent years. Along with this trend, an increasing number of cases of bearded dragons with leukemia as compared to other cancers have been reported from veterinary clinics and zoological institutions. Preliminary data collection from veterinary clinics and zoological institutions has confirmed this trend. Age, sex, benign versus malignant, mass location, type of animal, habitat, metastases, the presence of other tumors, and the outcome of the patient were collected. Data were compiled for analysis. There were 15 males, 11 females, and 14 animals where the sex was unknown. The average age at diagnosis was 68 +/- 45 months. There were 37 malignant neoplasms and 3 benign neoplasms. Leukemia was the most common neoplasm overall (n=7). Chromatophoromas were the most common benign neoplasm (n=3). Out of all the malignant cases, the most common survival times were 0.75, 1.50, and 6 months (average 5.75 months). Overall this study shows that malignant neoplasia is common in bearded dragons and that veterinarians should monitor these animals regularly for the development of neoplasia.
Idiopathic pulmonary fibrosis (IPF) is a form of interstitial lung disease characterized by scarring of lung tissue, which can cause respiratory failure and death. While FDA-approved drugs can slow disease progression, they cannot reverse IPF scarring. This lack of sufficient treatments demonstrates a need for new therapeutics that recover healthy tissue in IPF patients. Lung spheroid cells (LSCs) are derived from a heterogeneous population of human lung cells and have known regenerative properties. LSCs release nanoparticles called exosomes that share their therapeutic properties. Exosomes are 30-100 nm vesicles used in cell communication and marked for selective targeting of cells. LSC-derived exosomes reduce collagen build-up in IPF rodents and restore lung function. This study investigated how loading exosomes with miR-30a, a microRNA that reduces fibronectin and α-smooth muscle actin expression, affects tissue regeneration in the fibrotic murine lung. Lower expression of these proteins would reduce scarring in the lungs, and a combination of miR-30a with the therapeutic LSC-exosomes is expected to enhance tissue regeneration in the lungs of IPF mice. Normal expression levels of miR-30a were determined in both LSCs and mouse lung tissue using qPCR. Loaded and unloaded LSC-exosomes were characterized by nanoparticle tracking, western blotting, and TEM. Loaded exosomes were delivered to cultured LSCs to determine uptake efficiency in-vitro. A preliminary in-vivo experiment measured miR-30a delivery in the murine lung using qPCR. This study lays the groundwork for the development of a new treatment for patients with IPF.

Funding Source: N/A

Category: Regenerative Medicine
BROADENING THE SPECTRUM OF EXOTIC ANIMAL SPECIES NEOPLASIA: AN ANALYSIS OF ROLLINS LABORATORY CASES

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NCSU CVM (Smith, Duke, Harrison), ESCRA Database (Duke, Harrison), Rollins Animal Disease Lab (Trybus)

Currently, there is a lack of readily available data regarding neoplasia in exotic species. The Exotic Species Cancer Research Alliance’s (ESCRA) mission is to increase published data in this area through the use of their exotic tumor database. Our goal was to broaden the spectrum of neoplasia commonly found in exotic species using pathology reports from Rollins Animal Disease Laboratory. Rollins has over 6,000 cases during January 2011 – March 2019, and 416 of these are histopathologically confirmed cases of neoplasia in exotic animal species. This data was organized into preselected categories including species, age, sex, tumor type, location of tumor, tumor behavior, and treatment. We found that of these 416 cases, there were 4 animal orders, over 50 species, and more than 200 different histologic diagnoses within 12 different tumor classifications. The majority of tumors found were considered malignant, but only 20% of those tumors metastasized. This information will contribute to the ESCRA database to be able to gain a better understanding of prevalence and survival of exotic animals with cancer. By publishing more information about cancer in exotic species we hope to lay groundwork for future studies, as well as increase publicly available data regarding treatment, prognosis, and common types of neoplasia in exotic species.

Research Grant: none
Student Support: NC State VSP and BIVSP
Subject Category: Other (Oncology)
Complications due to orthopedic device-related infections (ODRI) result in high morbidity and large health-associated costs. *Staphylococcus aureus* is a common bacteria causing these infections due to its ability to create protein-based biofilms using host fibrin(ogen) and other plasma proteins. These biofilms are difficult to treat due to poor ability of antibiotic penetration of the biofilm along with altered microbial metabolism within the biofilm. This results in antibiotic resistance and chronic infection, which can lead to long-term antibiotic therapy, revision surgery, and death. The purpose of this study was to develop a model of ODRI that mimics orthopedic biofilms encountered within an articular environment. The hypothesis was that an in vitro orthopedic biofilm could be grown on orthopedic screws within joint synovial fluid. Two orthopedic screws (2.7 x 12 mm cortical screws) were placed in the first column of a 24-well ultra-low attachment polystyrene plate. Equine synovial fluid, obtained aseptically, was added to each well with screws, inoculated with *S. aureus* (1x10^6 CFU/ml), and allowed to incubate for a variable amount of time. Biofilm formation was qualitatively assessed with fluorescent microscopy. Results indicated an increase in biofilm formation on orthopedic screws incubated for 48 hours compared to 24 hours. Further improvement in biofilm density was noted with addition of exogenous human fibrinogen (200 mg/ml) at 24 hours following inoculation. These findings support the hypothesis that a model of orthopedic biofilm in equine synovial fluid can be achieved.

NCSU Veterinary Scholars Program, Stipend support: NIH 5T32 OD011130-13 (DWK) Cell Biology
SPINAL CORD AND DURAL SAC TERMINATION AND MORPHOMETRY IN DIFFERENT DOG BREEDS.

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Background- Termination of the spinal cord is developmentally complex and prone to congenital malformations. Despite breed differences in vertebral morphology, there is little information available on this region in different dog breeds.

Hypothesis/Objectives- Describe the location of spinal cord and dural sac termination and filum terminale internum length (FTIL) across dog breeds and identify influencing factors.

Animals- 120 dogs aged >1 year with normal lumbar magnetic resonance imaging (MRI) studies.

Methods- Blinded retrospective study. Vertebral location of spinal cord and dural sac termination were recorded from sagittal T2-weighted and HASTE lumbar MRIs, FTIL was measured on T2 images. Breed, weight, sex, and craniofacial classification were recorded. Vertebral location of terminal structures and FTIL were compared with weight, sex and craniofacial classification using multivariate logistic models.

Results- There were 42 breeds, with 32 brachycephalic, 79 mesaticephalic and 9 dolichocephalic dogs, 59 female, 61 male and median weight: 23.3kg (4.1 to 62kg). Sex and craniofacial classification were not related to vertebral level of spinal cord and dural sac termination or FTIL while weight was (P = 0.0009, 0.0037 and < 0.0001 respectively). Boston terriers, Corgis and CKCS were outliers compared with similar weight dog breeds, with more cranial spinal cord termination in Boston terriers, and more caudal in Corgis and CKCS.

Conclusions and Clinical Significance- The location of spinal cord terminal structures is affected by weight, but not sex or cranial morphology. It does appear to differ in breeds known to have congenital skeletal malformations. These deserve further evaluation in larger cohorts.

Funding Sources: Not Applicable
Subject Category: Neurosciences
IN VIVO RADIATION DOSE-RESPONSE RELATIONSHIPS FOR A SYNGENEIC ORTHOTOPIC MURINE MODEL OF ORAL SQUAMOUS CELL CARCINOMA

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Growth of Mouse Oral Cancer 2 (MOC2) cells in mice provides a powerful tool for studying oral squamous cell carcinoma (OSCC). Because OSCC is commonly treated with radiotherapy, the objective of this study was to characterize the radiation dose-responsiveness of orthotopically grown MOC2 tumors in their syngeneic host. Female C57Bl/6 mice underwent intralingual injection of 4x10^5 MOC2 cells. Four days later, the resultant tongue tumors were irradiated (5 daily fractions of 0, 4.5, 5.5, 6.5, or 7.5 Gy; N = 6 mice per dose level) using an experimental irradiator (XRAD320XL). Mice were monitored daily and euthanized when they had lost more than 20% body weight and presented inability to prehend food or water, dyspnea, facial wiping indicating pain, and if tumors reached measurement endpoint (6 mm or wider). Necropsy was performed to evaluate for metastasis. Tumors developed in 29/30 mice (97%). Subjectively, tumors were difficult to measure, and often associated with necrosis of adjacent tongue tissue. In sham-irradiated mice, tumor growth necessitated euthanasia by day 9. At 22.5 Gy, there was minimal initial tumor regression followed by progression by day 11 post-IR. Higher radiation doses did not cure tumors but did further delay tumor progression and prolong survival. One mouse had equivocal regional lymphadenomegaly, but necropsy identified no other evidence of metastasis in any mouse. Despite these limitations, these results indicate a clear radiation dose-response relationship, and this work lays the groundwork for future experiments that are designed to test novel combinatorial therapies to improve the care of HNSCC patients.

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Subject category: Cell Biology
Macrophages are innate immune cells that adopt a wide variety of phenotypes, the extremes of which are pro-inflammatory (M1) or anti-inflammatory (M2). Tumors reprogram macrophages directly or through the tumor microenvironment (TME) to induce an immunosuppressive phenotype permissive to tumor survival and growth. We hypothesized that canine osteosarcoma cells would induce the anti-inflammatory M2 macrophage phenotype. Our aims were to 1) determine whether canine osteosarcoma cells secrete signals that alter gene expression in canine macrophages, and 2) determine whether pre-stimulation of osteosarcoma cells with interferon-gamma (IFNg), a cytokine in the TME, would alter this effect. Abrams osteosarcoma cells were cultured in media for 24 hours to generate conditioned media (Abrams-CM). Canine macrophage-like DH82 cells were incubated with filtered Abrams-CM for 24 hours. Relative expression of macrophage M1 genes (TNFa, CXCL10) and M2 genes (TGM2, CD23) was measured by quantitative PCR (qPCR). We found that Abrams-CM significantly upregulated macrophage expression of TNFa and CXCL10 but had no effect on TGM2 or CD23. Compared to Abrams-CM, CM from IFNg-treated Abrams cells had no additional effect on macrophage expression of TNFa or CD23. In stark contrast, CM from IFNg-treated Abrams cells induced a 1000-fold increase in CXCL10 and 5-fold increase in TGM2. Together, these data show that canine osteosarcoma cells induce a pro-inflammatory, rather than an anti-inflammatory phenotype in macrophages, and that pre-stimulation with IFNg dramatically alters this effect. These studies begin to tease apart the complex signaling between osteosarcoma cells and macrophages, with potential implications for the treatment of canine osteosarcoma.

Primary category: Immunology