ErbB2 (HER2) overexpression is detected in 20-30% of all breast cancer cases, and is associated with higher probability of recurrence and poorer prognosis. Anti-ErbB2 therapy, including trastuzumab (Herceptin), as monotherapy or in combination with doxorubicin, is an effective method of treating HER2 positive breast cancer. Unfortunately, while these agents work synergistically as a treatment, they also cause increased cardiotoxicity. A cardiac-specific ErbB2 overexpressing mouse model was created to study the role of ErbB2 in cardiac function and protection against doxorubicin-induced cardiotoxicity. One striking characteristic of this mouse model is significant cardiac hypertrophy. Additionally, the hearts of the transgenic mice have an increased total protein ubiquitination. We hypothesize that a possible mechanism of ErbB2 overexpression-induced hypertrophy is decreased protein degradation via autophagy. To study ErbB2 effects on autophagy, 8 weeks old transgenic and wild type male mice were treated with rapamycin, chloroquine or vehicle daily. Rapamycin induces autophagy by inhibiting mTOR (a downstream effector of ErbB2). Chloroquine inhibits autophagy by preventing phagosomal-lysosomal fusion. Animals were sacrificed after 20 days and the heart, liver, and kidneys were saved for the molecular and histological studies. Heart weights were significantly decreased in the rapamycin treated transgenic animals, compared to the wild types (~20% average decrease). Immunoblotting showed a decrease in ubiquitinated proteins in the transgenic mice hearts, after rapamycin treatment, compared to the wild types.

In conclusion, ErbB2 overexpressing mice present with significant cardiac hypertrophy, which is partially reversed by autophagy induction with mTOR inhibitor rapamycin. Future experiments are planned to explore the role of autophagy in cardiac hypertrophy, and the regulation of autophagy by ErbB2, as well as effects of doxorubicin treatment on autophagy.
Long-term Outcome Following Placement of an Ameroid Ring Constrictor For Treatment of a Congenital Extrahepatic Portosystemic Shunt in Dogs

Emily Falls, Milan Milovancev, Chad Schmiedt

Surgical placement of an ameroid ring constrictor (ARC) is one of the most common means of treatment of congenital extrahepatic portosystemic shunts (CEHPSS). Currently, the longest reported median period of post-operative follow-up is 3 years, and despite the common use of ARCs, there is no data on long-term outcome following this procedure. The purpose of the study described here is to provide data regarding long-term and lifetime outcome of dogs that underwent ARC placement for treatment of a CEHPSS. In addition, correlations between pre- and peri-operative factors and long-term outcome were evaluated.

The medical records of all dogs undergoing surgical treatment of a CEHPSS via ARC placement at the University of Georgia, University of California at Davis, and North Carolina State University between the years of 1995 and 2002 were reviewed. Signalment, pertinent laboratory data, and neurologic status at the time of diagnosis were recorded. Surgical ARC placement was performed, and the date of surgery and any clinically relevant information was collected from the surgical report. Attempts were made to contact each client and/or referring veterinarian and collect long-term follow-up information. Based on the date of the patient’s last known status, follow-up information was categorized as short-term, medium-term, or long-term. Information regarding the status of some dogs was extracted from previously conducted research. The outcome of each case was determined to be “poor,” “good,” or “excellent. Statistical analysis was performed to reveal associations between each pre- and peri- operative factor and the dog’s long-term outcome.

Of the 206 records reviewed, long-term follow-up (defined as follow-up collected at more than 36 months post-operatively) was available for 56 dogs. Of these 56 dogs, the outcome of 47 was categorized as excellent, 4 as good, and 5 as poor. Increasing lengths of post-operative survival were positively correlated with the probability of an excellent outcome (odds ratio OR = 1.002 (95% CI 1.000-1.003), p=0.0087). There was also a significant positive correlation between medium-term and long-term outcome (r=0.59, p<0.0001). Having a negative (<15%) nuclear scintigraphy at 4-14 weeks post-operatively increased a dog’s odds of an excellent outcome by 11 times. Being intact significantly increased the probability of surviving the short-term period, while an increase in pre-operative WBC count decreased this probability.

Based on these results, it can be expected that the majority of dogs who are clinically normal three years following ARC placement surgery will suffer no further effects of this condition and can be considered “normal.” This likelihood is predicted by a negative nuclear scintigraphy outcome when performed 4-14 weeks post-operatively.
EVALUATION OF THE EFFECTIVENESS OF ANALGESIC REGIMENS IN A UNIVERSITY INTENSIVE CARE UNIT

Clara E. Moran and Erik H. Hofmeister

Despite the broadly accepted importance of pain control in veterinary patients, little research has been done to assess the efficacy of standard analgesic regimens in a clinical setting. The authors of this paper hypothesized that some dogs in a university ICU still experience an unacceptably high level of pain. To assess this hypothesis, canine patients in the University of Georgia’s Teaching Hospital ICU were pain scored twice a day at randomly determined time points, using three different scales: simple descriptive scale, visual analog scale, and Glasgow pain scale. Information about each patient’s signalment, condition, and analgesic treatments was recorded at the same time, and the data was analyzed to identify any statistically significant risk factors.

A total of 629 assessments were performed, and overall 20% of patients were identified as being painful. This number was consistent regardless of the time of day or day of week, and no aspects of the patient’s signalment or condition were significant predictors of whether the patient was classified as painful or not. Analysis of analgesic regimens revealed that dogs treated with tramadol, butorphanol, or no analgesic were significantly less likely to be classified as painful than dogs treated with other protocols, but this most likely reflects the fact that these animals were judged to be less painful initially, and therefore only in need of weak or no opioids. A comparison of the three scoring methods revealed greatest agreement between VAS and SDS, and that the order in which each score was collected did not impact the scores received.
Sturgeon, commonly known for and valued for their precious roe, are suffering great losses in the wild due to habitat change and overfishing. To better protect these elusive fish, gender identification is a key method practiced in the field in order to determine critical spawning habitats as well as habitats crucial to the growth and development of juvenile sturgeon. As a result of the endangerment status of many species of sturgeon, aquaculture, therefore, serves as the major producer of sturgeon for the caviar industry. As such, gender identification is essential for the production of caviar, and early gender identification has become increasingly desired in order to successfully raise greater numbers of female sturgeon. Currently, most hatcheries can determine gender in 4 to 5 year old Siberian sturgeon (Acipenser baerii) using primarily endoscopy.

In the present study, Siberian sturgeon (Acipenser baerii) were selected to undergo ultrasonographic and endoscopic evaluations for gender identification. University of Georgia’s sturgeon hatchery served as the sampling site for the study. The goal of the study was to accurately determine gender in 3 year old Siberian sturgeon using non-invasive ultrasonography and compare this method’s effectiveness with minimally-invasive endoscopy. A total of 120, 3 year old Siberian sturgeon were anesthetized and evaluated using ultrasonography and endoscopy, and had a 2.5mm³ gonadal biopsy taken to confirm gender histopathologically. Histopathology confirmed the gender for 112 fish. Histopathology and endoscopy agreed on the gender of 96 fish; ultrasonography agreed on 90 fish. However, ultrasonography was found to be a faster method for gender identification than endoscopy. The ultrasonographer and the endoscopist were timed while examining the fish, and out of the 16 fish evaluated, the ultrasound showed gonads immediately while the ultrasonographer was able to determine gender 15 seconds, on average, faster than the endoscopist.

The results indicated that ultrasonography is an effective and fast imaging technique to determine early gender identification in sturgeon, but is not as accurate as endoscopy. This study also has applications for free ranging fish. Since many species of sturgeon are threatened or critically endangered, the ability to view gonads in fish species can reveal pertinent biological information that can lead to better management and recovery plans.
Mammary cancer is a leading cause of death in female dogs and the fifth cause of death in women. Abnormal chromosome number, or aneuploidy, is an identifying characteristic of cells that have undergone malignant neoplastic transformation. An altered epigenetic composition has been shown to play a role in genomic instability and consequently the onset of tumor formation and malignant progression. We hypothesize that chromatin remodeling proteins are abnormally regulated in an established canine mammary gland cancer cell line. Using live cell imaging, we have made novel attempts to detect abnormal chromosome segregation in actively dividing cells. Immunofluorescent microscopy was applied to detect the subcellular localization of chromatin remodeling proteins. We provide novel evidence that poly (ADP-ribose) polymerase (Parp-1) co-localizes with CREST signals at the kinetochores in canine mammary gland cells. Localization may be cell cycle dependent. Rampant aneuploidy was detected in the cancer cell line. Moreover, micronuclei formation consisting of whole missegregated chromosomes and acentric chromosome fragments were observed. Chromosome breaks were detected using γH2AX. Using real-time PCR, Parp-1 gene expression will be compared across canine cancer and normal mammary gland cell lines. Protein levels will be measured by Western Blot. In addition, Rad51 expression levels will be analyzed due to its clinical relevance and role in DNA repair by homologous recombination. Tissue specimens of varying malignancy will be compared to match the epigenetic profile with the degree of progression. These molecular markers could potentially be used in the early detection of cells that may lead to tumor formation as well as insight into treating or managing clinical cases. Understanding the mechanisms that cause chromosome instability, and consequently aneuploidy, is a step towards designing drugs that target these processes. In a one health initiative, findings could have immediate application to veterinary medicine and future applications to human medicine.
Trilostane is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, which is essential in adrenocorticoid synthesis and is employed as a therapy for hyperadrenocorticism (HAC) in dogs. Adrenal cortisol reserve is closely monitored in trilostane-treated patients to detect and avoid iatrogenic hypoadrenocorticism. The ACTH stimulation test, commonly used for monitoring, quickly becomes time consuming and expensive for the client. Cook et al (2010) demonstrated a baseline cortisol cut-off value that could predict adequate post-stimulation cortisol for HAC dogs on trilostane therapy. However, they excluded HAC dogs with comorbid conditions and their negative predictive value was less than 100%. We hypothesize that a baseline cortisol cut-off point exists which excludes the risk of hypoadrenocorticism with 100% negative predictive value. Additionally, we predict that such a cut-off can be used for HAC dogs with comorbid conditions.

Medical records were evaluated for all dogs who had undergone an ACTH stimulation test. Inclusion factors for this study were: patients diagnosed with HAC, stimulation tests that used only the intravenous form of synthetic ACTH, stimulation tests that evaluated only baseline and 1-hour post-ACTH samples analyzed by a reference laboratory, and treatment with brand name trilostane (Vetoryl®) at the time of the stimulation testing. Exclusion factors used in this study were patients who received mitotane or compounded trilostane for their HAC treatment. Data extracted from medical records for each ACTH stimulation included signalment, comorbid conditions, results of hematological and biochemical analyses, presence or absence of clinical control of HAC, dose of trilostane at the time of stimulation testing. Eight patient records have been enrolled to date reflecting 20 total ACTH stimulation tests. Two hundred twenty medical records remain to be screened.

After all the records are enrolled, dogs will be stratified into HAC only (Group A) and HAC with comorbid conditions (Group B). The relationship between baseline and post-stimulation cortisol level will be calculated by linear regression for Group A dogs. A ROC will be created, and the cut-off value will be determined which predicts adequate adrenal reserve (post-stimulation cortisol >2.0 ug/dL) with 100% negative predictive value. For Group B, the relationship between baseline and post-stimulation cortisol level will be calculated by linear regression. The negative predictive value of the cut-off value identified in Group A will be determined for Group B dogs. If the negative predictive value is not 100%, a new ROC will be created for Group B dogs, and the cut-off value predictive of adequate adrenal reserve with 100% negative predictive value will be determined for this group.
“INDOLEAMINE 2,3-DIOXYGENASE INHIBITION ENHANCES THE MEMORY IMMUNE RESPONSE TO INFLUENZA INFECTION”

SAGE, L.K., FOX, J. M., TOMPKINS, S.M., TRIPP, R.A.

Indoleamine 2,3-dioxygenase (IDO) is an immunomodulatory enzyme produced by epithelial cells and immune regulatory cells that functions to catabolize tryptophan through the kynurenine pathway. The kynurenine metabolites can attenuate T cells responding to antigen and reduce inflammation. IDO is upregulated in response to influenza virus infection, but its effect on the immune response in the context of viral infections remains to be understood. In this study, we evaluated the effects of IDO on the memory immune response to influenza virus infection by manipulating the effects of IDO during influenza specific memory T cell formation using a competitive inhibitor of IDO, 1-methyl tryptophan (1MT). Mice were intranasally infected with Influenza A/Hong Kong/1/68 x A/Puerto Rico/8/34 (X31; H3N2), with 1MT or placebo treatment. After the X31 infection was resolved, mice were challenged with Influenza A/Puerto Rico/8/34 (PR8; H1N1). The rationale for using these viruses is that X31 and PR8 share the same internal genes which T cells recognize, but are serologically distinct, thus memory T cell responses can be evaluated and exclude the effect of antibodies. Lymphocytes isolated from the mediastinal lymph node (MLN), and lungs (by bronchoalveolar lavage) after PR8 challenge were analyzed by flow cytometry for cell types present, states of cell activation, Th1/Th2 cytokine expression, and influenza antigen specific CD8+ T cell responses. Mice with IDO inhibition (treated with 1MT) had more robust T cell responses indicated by higher levels of effector (CD44hiCD62Llo) T cell trafficking to the lungs, higher level of Th1 response, and a shift in CD8+ T cell (MHC Class I restriction) immunodominance, compared to placebo group. This study demonstrates that IDO modulates the memory T cell response to influenza virus infection, and has important implications in mucosal immunology and vaccination strategies which may employ IDO inhibitors to enhance immune response to vaccines.
USE OF THE ALPHATRAK GLUCOMETER FOR IDENTIFYING SEPTIC PERITONITIS
Verlander, Lindsey Lane; Koenig, Amie; Brainard, Ben

A diagnosis of septic peritonitis is supported by identifying a difference of ≥20 mg/dl between whole blood and peritoneal fluid glucose concentrations. Glucose concentration is routinely measured using hand-held glucometers, which may or may not be accurate, depending on the cellularity of the sample. The AlphaTRAK glucometer was marketed as being more accurate for companion animals than other glucometers because it utilizes an algorithm which accounts for species differences in the red cell-bound versus dissolved glucose. Severe anemia falsely elevates AlphaTRAK results. Since most septic effusions have a low hematocrit, the peritoneal fluid AlphaTRAK values may be falsely elevated and fail to indicate septic peritonitis.

The purpose of this pilot study was to determine if the AlphaTRAK can be used to correctly identify septic peritonitis by using the traditionally cited 20 mg/dl glucose difference between blood and peritoneal effusion. We hypothesized that the AlphaTRAK would fail to identify all septic peritonitis patients using the glucose difference between whole blood and peritoneal effusion but would more accurate using plasma and peritoneal effusion.

Sixteen septic and 15 non-septic peritoneal effusions were tested. The AlphaTRAK was used to measure glucose in whole blood (WB), plasma (Pl), peritoneal effusion (PE), and peritoneal effusion supernatant (PS). The WB-PE difference yielded 50% sensitivity and 73% specificity, Pl-PE yielded 93.4% sensitivity and 47% specificity and Pl-PS yielded 100% sensitivity and 53% specificity for identifying septic peritonitis. In contrast, the NOVA biochemical analyzer yielded 83% sensitivity and 65% specificity for WB-PE.

Using the traditional 20 mg/dl glucose difference as the benchmark for identifying septic peritonitis, the AlphaTRAK failed to identify 50% of the septic peritonitis patients when using native WB and PE samples. Using Pl-PS maximized sensitivity.
KINASE SCREEN FOR THE CHEMOPREVENTIVE ENHANCEMENT OF BEXAROTENE IN BREAST CANCER

Eve Winkleman, Ivan P. Uray, Powel Brown

Bexarotene, a vitamin D derivative that is currently FDA approved as an antineoplastic agent in T cell lymphoma, has also been shown to be highly effective in the prevention of breast cancer in ErbB2 transgenic mice. This prevention, however, is not one hundred percent effective. It is known that Bexarotene activates a kinase pathway, but it is not known precisely which, nor which kinases are involved. Thus, a knockdown kinase screen was conducted on the human kinome in order to identify kinases that, when knocked down, either enhanced or inhibited the activity of Bexarotene. This study is currently ongoing, but preliminary results have shown several hits in the kinome, in particular PKCε (Protein Kinase C Epsilon).
ANTAGONIZING EFFECTS OF DOXAPRAM ON ACEPROMAZINE SEDATION
Mark Zapata, Erik Hofmeister

The purpose of this study was to evaluate the effectiveness of two doses of doxapram as an antagonist to acepromazine sedation in dogs. The hypothesis was that the dogs administered the 2.5mg/kg dosage of doxapram after 0.05mg/kg acepromazine would decrease the sedation scores more quickly and significantly than the group administered the 1.25mg/kg dosage and the dogs in the control group.

Dogs were randomly assigned to one of three treatment groups. Group 1 (control) received acepromazine (0.05mg/kg) administered intramuscularly followed 30 minutes later by a saline solution (0.9% NaCl) administered intravenously. Group 2 received acepromazine (0.05mg/kg) administered intramuscularly followed 30 minutes later by doxapram (1.25mg/kg) administered intravenously. Group 3 received acepromazine (0.05mg/kg) administered intramuscularly followed 30 minutes later by doxapram (2.50mg/kg) administered intravenously. Each dog was initially visually examined from outside its cage. The observer then entered the cage to allow interactive behaviors and restraint to be evaluated. The response to noise was then evaluated while the observer remained in the cage. This procedure was done uniformly for all the dogs in the study. Sedation scores were obtained at 0, 15 and 30 minutes after acepromazine administration and at 5, 15 and 30 minutes after doxapram administration.

There were no significant differences among groups for baseline sedation (P=0.97) or sedation after 30 minutes (P=0.44). Sedation scores decreased significantly after doxapram (6.4 to 4.3 in 1.25; 6.3 to 4.9 in 2.5; P<0.003) but not after saline (7.4 to 6.3; P>0.05). There was not a significant difference between the 1.25mg/kg dose and the 2.50mg/kg dose. Male dogs (n=5) that received the 2.50mg/kg panted immediately following injection.

The results suggest that the administration of doxapram does have significant antagonizing effects on acepromazine sedation. The dogs that received doxapram had significantly lower sedation scores following administration that those that received only a saline solution. The results also suggest that a 1.25mg/kg is sufficient to antagonize acepromazine sedation because there was no significant difference in the sedation scores following the 1.25mg/kg and 2.50mg/kg doses. There were also no adverse effects following the administration of the 1.25mg/kg dosage as there were following the 2.50mg/kg dosage.
ANTIBODIES TO THE CENTRAL CONSERVED REGION OF RESPIRATORY SYNCYTIAL VIRUS (RSV) G PROTEIN BLOCK RSV G PROTEIN CX3C-CX3CR1 BINDING AND CROSS-NEUTRALIZE RSV A AND B STRAINS

Youngjoo Choi, Caleb Mason, Les B.N. Jones, Jackelyn Crabtree, Patricia Jorquera, and Ralph A. Tripp

Respiratory syncytial virus (RSV) is a primary cause of severe lower respiratory tract disease in infants, young children, and the elderly worldwide, and despite decades of effort, there remains no safe and effective vaccine. RSV modifies the host immune response during infection by CX3C chemokine mimicry adversely affecting pulmonary leukocyte chemotaxis and CX3CR1 RSV-specific T cell responses. This study investigated whether immunization of mice with RSV G protein polypeptides from strain A2 could induce antibodies that blocked G protein CX3C–CX3CR1 interaction of both RSV A and B strains. The results show that mice immunized with RSV A2 G protein polypeptides generate antibodies that block binding of RSV A2 and B1 native G proteins to CX3CR1, and that these antibodies effectively cross-neutralize both strains of RSV. Our findings suggest that vaccines that induce RSV G protein CX3C-CX3CR1 blocking antibodies may provide a disease intervention strategy in the efforts to develop safe and efficacious RSV vaccines.
DEVELOPMENT OF STANDARDIZED MOLECULAR DIAGNOSTIC METHOD FOR ANTIGENICALLY CHARACTERIZING INFECTIOUS BURSAL DISEASE VIRUSES ACROSS THE GLOBE BY USING FTA CARDS AND REVERSE GENETICS

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Infectious bursal disease is an immunosuppressive disease in young chickens caused by infectious bursal disease virus (IBDV). IBDV is a non-enveloped icosahedral virus containing a bisegmented double-stranded RNA genome, segment A and B. This virus is ubiquitously present in poultry flocks and can cause substantial economic losses. IBDV destroys immature B lymphocytes by lytic infection leading to humoral immunosuppression. Thus, chickens become more susceptible for facultative pathogens and vaccinations against other pathogens result in a suboptimal seroconversion which leads to vaccination failures. Being an RNA virus, IBDV can quickly evolve due to presence of neutralizing antibodies which might result in an antigenic drift. This usually results in the emergence of antigenically different strains in the field. Thus, the selection of an appropriate vaccine candidate for IBDV becomes more complicated for vaccine developer and poultry companies. In previous studies, we characterized various strains of IBDV from commercial US poultry flocks by applying reverse genetics. In the present study, we were interested in determining the antigenicity of IBDV field strains from outside US. To this end we combined FTA® cards with IBDV reverse genetics for antigenic characterization. FTA® cards were used for a safe transport of the genetic material without importing infectious material across country borders. To this end a protocol was developed which enabled us to isolate RNA from FTA cards for our experiments. Specific primers for the amplification of cDNA were appropriately selected based on the results of sensitivity assay conducted with different primer pairs. The selected primer pairs were used to amplify a cDNA fragment of the genomic segment A which encodes for the part of the viral protein 2 folding the neutralizing epitopes. The amplified cDNA was cloned into the established IBDV reverse genetics system and the obtained nucleotide and amino acid sequences were analyzed by bioinformatics. Using the IBDV reverse genetics systems the antigenicity was determined after transfection of cRNA. The expressed VP2 protein was analyzed with a panel of monoclonal antibodies which characterize known neutralizing epitopes of IBDV. It was observed that the results of the sequence analyses by Blast search (blastn, blastp) did not correlate with the antigenic phenotype as determined after RNA transfection by using the panel of monoclonal antibodies. In conclusion, by using this standardized molecular diagnostic approach, IBDV can be antigenically characterized from bursal samples across the globe without sending the infectious virus across country borders.
INTERFERON LAMBDA UP-REGULATES THE EXPRESSION OF IDO1 IN ALVEOLAR EPITHELIAL CELLS

Julie M. Fox, Leo K. Sage, S. Mark Tompkins, and Ralph A. Tripp

Influenza infection stimulates a significant increase in the level of indoleamine 2, 3-dioxygenase (IDO) activity in the lung parenchyma. IDO is the first and rate limiting step in the kynurenine pathway where tryptophan is broken down into kynurenine and other metabolites. Multiple cell types express IDO including dendritic cells, macrophages, and epithelial cells. While up-regulation of IDO in dendritic cells and macrophages is primarily mediated by IFNγ, the up-regulation and role of IDO expression in epithelial cells during an influenza infection has not been thoroughly examined. It is well known that IFNγ and to a lesser extent IFNα/β are stimulators of IDO expression; however, interferon lambda is the main anti-viral cytokine produced by epithelial cells during influenza infection. This is in part due to the ability of the influenza NS1 protein to suppress the type I interferon response. In this study, we investigated the role of interferons in the regulation of IDO expression. The expression of the IDO1 and IDO2 was assessed in a mouse type II pneumocyte cell line (MLE-15) during influenza infection followed by measurement of interferon production. Influenza dramatically increased IDO1 during infection which correlated with peak interferon lambda production. Following stimulation with recombinant IFNλ, IDO1 mRNA was up-regulated. These results provide a role of IFNλ in the stimulation of IDO1 during an influenza infection allowing for future studies to further elucidate the role of IDO in epithelial cells during infection.
MODELING THE INFLUENCE OF HOST COINFECTION ON TRANSMISSION IN A VECTOR-BORNE PATHOGEN

Hou-Ming L. Fung and Nicole L. Gottdenker

Understanding the ecology of diseases is fundamental for targeting disease transmission and control, particularly for vector-borne pathogens that infect a variety of hosts. Most wild and domestic reservoir hosts are coinfected with a community of parasites and/or pathogens, and these coinfections may influence the transmission dynamics for a vector-borne disease. The objective of this study is to evaluate how parasite coinfection influences individual susceptibility by examining the association between micro and macroparasites. A mathematical model was built to examine the association between *Trypanosoma cruzi*, a vector-borne zoonotic protozoan and causative agent for Chagas disease, and intestinal helminths in canine hosts. The model created for the *T. cruzi* transmission shows that intestinal helminths infection may potentially increase the transmission of *T. cruzi* between hosts. By building a predictive model of disease transmission for this system, this project could contribute to control efforts aimed at managing *T. cruzi* and other vector-borne parasite and pathogen transmission.
Runting and Stunting Syndrome (RSS) is an enteric disease of unknown etiology affecting young meat type chickens (broilers). Weight suppression and lack of flock uniformity associated with diarrhea are observed with RSS. Cystic enteropathy in the small intestine is a hallmark lesion observed by microscopic examination. Although descriptions of RSS date back to the 1970s, the etiologic agent(s) has yet to be determined. However, the disease is reliably reproduced using filtered intestinal homogenates from RSS affected broilers, implying a viral etiology. Based on subtractive metagenomic studies, four viruses were identified which might play a role in the etiology of RSS. The genetic information of three different chicken astroviruses (CkAstv) and a chicken parvovirus (CkParv) was cloned, and the viral cDNA used for development of negative-sense riboprobes for in situ hybridization (ISH). One day old commercial broiler chickens were exposed to RSS-contaminated litter, and one group was placed on fresh wood shavings. Five chickens were obtained at each day from day 1 to 5 p.i., and formalin-fixed paraffin-embedded tissues of the duodenal loop were analyzed by two approaches: ISH and microscopic examination. Nucleic acids from two CkAstvs were identified by ISH only in the villous epithelial cells. Interestingly, the nucleic acid of the third CkAstv was observed in both the crypt as well as villous epithelial cells. Cystic enteropathy and location of viral RNA, as observed by ISH signals, were progressive and increased with age but were not always associated with each other. All tissues were negative for the CkParv riboprobe. These results strongly suggest the involvement of chicken astroviruses in the etiology of RSS. Furthermore, the presence of ISH signals in the crypt region of the duodenum implies that a new CkAstv might be closely associated with the formation of the hallmark lesion for RSS, the cystic enteropathy.
EPIDEMIOLOGY OF *MYCOBACTERIUM CHELONAE*

Jonathan Lane, Susan Sanchez

*Mycobacterium chelonae* causes fatal infections in fish, birds, mammals, and reptiles and is opportunistic pathogen of humans. It is pervasive in the environment, putting it in contact with many different host species. Many case studies have looked at *M. chelonae* infection in a single host or outbreak among a single species but there has been no research done to determine the relatedness of pathogenic *M. chelonae* between different hosts. *hsp65* sequencing has been of value for the identification of *M. chelonae* complex species and approaches based on the sequencing of other sections of the genome, *rpoB* and the 16s-23s internal transcribed spacer region (ITS), have shown promise as an identification tool. Repetitive, sequence-based PCR (rep-PCR) has been used to separate strain level mycobacteria and was recently automated (DiversiLab system; BioMérieux, Melbourne, Victoria, Australia) for ease of use.

We obtained 30 clinical *M. chelonae* complex isolates from various reptile, fish, and mammal species as well as humans. PCR was performed to isolate the *hsp65* and *rpoB* genes, and the ITS region and subsequent sequencing of the DNA from these loci and use of the Diversilab system led to the identification of the 30 clinical *M. chelonae* complex isolates.

Phylogenetic trees reveal relatively homogeneous gene sequences in all 3 loci sequenced. Rep-PCR produced the most heterogeneity between strains but did not display host class separation.

Results indicate that strains of *M. chelonae* show little variance, no matter the host from which they were isolated. Additionally, comparison of identification techniques demonstrates that sequencing of the *hsp65* gene remains the most reliable way to distinguish *M. chelonae* complex isolates from each other and that the Diversilab system is not an appropriate way of typing *M. chelonae* complex isolates.
Influenza A virus causes seasonal epidemics and periodic pandemics threatening the health of millions of people each year. Vaccination is an effective strategy for reducing morbidity and mortality, and in the absence of drug resistance, the efficacy of chemoprophylaxis is comparable to that of vaccines. However, the rapid emergence of drug resistance has emphasized the need for new drug targets. Knowledge of the host cell components required for influenza replication has been an area targeted for disease intervention. In this study, the human protease genes required for influenza virus replication were determined and validated using RNA interference approaches. The genes validated as critical for influenza virus replication were ADAMTS7, CPE, DPP3, MST1, and PRSS12, and pathway analysis showed these genes were in global host cell pathways governing inflammation (NF-κB), cAMP/calcium signaling (CRE/CREB), and apoptosis. Analyses of host microRNAs predicted to govern expression of these genes showed that eight miRNAs regulated gene expression during virus replication. These findings identify unique host genes and microRNAs important for influenza replication providing potential new targets for disease intervention strategies.
PROTECTION AGAINST H5N1 VIRUS CHALLENGE BY IMMUNIZATION WITH RECOMBINANT PIV5 ENCODING THE H5 HEMAGGLUTININ GENE.

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Highly pathogenic avian influenza virus (HPAI) and the H5N1 subtype in particular, pose a formidable pandemic threat. Current HPAI vaccine candidates suffer from poor immunogenicity, and there are challenges associated with sufficient production and distribution. Human parainfluenza virus 5 (PIV5) provides an appealing approach for live virus vectored vaccines. Using reverse genetics techniques, we have inserted the HA gene of A/Vietnam/1203/04 (H5N1) into the PIV5 genome with the goal of testing the efficacy and mechanism of protection of recombinant PIV5-H5 vaccine vectors. The natural gradient of mRNA translated in PIV5 is dictated by the proximity of the gene to the 3' UTR. Thus, we tested expression and immunogenicity of H5 vaccine vectors where the HA gene is inserted in distinct locations in the PIV5 genome. The H5 HA was inserted between the SH and HN genes (ZL46), and between the HN and L genes (ZL48) in the PIV gene order: 3' - NP – V/P – M – F – SH – HN – L – 5'. Vero cells infected with these constructs expressed H5 HA on the cell surface. Using a BALB/c mouse model, we show that both PIV5-H5 constructs induce H5-specific serum antibody responses, and vaccination with these constructs reduces weight loss and lung virus titers upon A/VN/1203/04 challenge. ZL46 conferred robust protection, comparable to vaccination with whole inactivated A/VN/1203/04, or protection conferred following a sub-lethal infection with recombinant A/VN-PR8 infection. The protection conferred by passive transfer of IgG from vaccinated mice indicates that neutralizing antibodies are associated with vaccine efficacy. Future and ongoing studies include further exploration of mechanism, cross-clade protection, route of administration, and rPIV5 constructs expressing other influenza genes such as neuraminidase.

This study was supported by Influenza Pathogenesis & Immunology Research Center (IPIRC); NIH contract HHSN266200700006C.
Influenza A viruses are responsible for annual epidemics and intermittent pandemics. Annual vaccination is the most effective strategy for preventing influenza. Unfortunately, there is insufficient vaccine manufacturing capacity to allow for the recommended immunization coverage. Despite egg-based influenza vaccine production being well established, there are influenza strains that do not propagate in eggs, or are lethal to eggs, and often the hemagglutinin (HA) is poorly antigenic. In this study, we investigated a novel vaccine strategy comprising cell-based expression of HA and the fusion (F) protein of respiratory syncytial virus (RSV). The approach in this study takes advantage of the known TLR4 interaction of RSV F protein, and its feature as the major target antigen known to induce protective immunity to RSV infection. Mice were vaccinated with either the HA protein alone or in combination with the F protein. The F protein was either directly conjugated to the HA proteins or given in equal concentration with the HA protein. The results show the F protein acts as a molecular adjuvant to enhance the anti-HA immune response and protecting from influenza virus challenge, while also inducing a potent anti-F protein response protecting from RSV challenge. Interestingly, preliminary evidence suggests the possibility of cross-reactive B cell epitopes between HA and F protein, a feature that is being explored and may be potentially discussed. These results show that cell-based HA vaccines may be enhanced by the contribution of RSV F protein.
RESPIRATORY SYNCYTIAL VIRUS ALTERS THE EXPRESSION OF HOST MIRNAS REGULATING MULTIPLE PATHWAYS

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Respiratory syncytial virus (RSV) causes substantial morbidity and life-threatening lower respiratory tract disease in infants, young children, and the elderly. Understanding the host response to RSV infection is critical for developing disease intervention approaches. The role of microRNAs (miRNAs) in post-transcriptional regulation of host genes responding to RSV infection is unknown. In this study, we show that RSV infection of type II lung epithelial cells induces five miRNAs (let-7f, miR-24, miR-337-3p, miR-26b and miR-520a-5p) and represses two miRNAs (miR-198 and miR-595). RSV G protein was found to be a major inducer of let-7f. Luciferase-UTR reporters and miRNA mimic and inhibitors validated a subset of predicted target genes for let-7f, specifically showing let-7f governance of cell cycle regulators (CCND1, DYRK2 and ELF4), a chemokine gene (CCL7), and suppressor of cytokine signaling 3 (SOCS3) genes. These results show that RSV G protein affects let-7f mediated regulation of multiple gene networks during infection, a feature that may contribute to cell cycle regulation and immune modulation to facilitate replication or persistence.
ANALYSIS OF MEIOTIC SPINDLE CONFIGURATIONS AND CHROMOSOME COMPLEMENTS IN EQUINE OOCYTES

Claudia Baumann, Maria M. Viveiros, Dirk K. Vanderwall and Rabindranath De La Fuente

Aneuploidies are most commonly caused by chromosome segregation errors during meiosis in female germ cells and result in early embryonic loss, congenital birth defects or infertility. Evidence suggests the involvement of several risk factors in the etiology of aneuploidy, such as abnormalities in the establishment of a functional epigenetic landscape at centromeric chromosome domains, disruptions in spindle structure and chromosome-microtubule attachment or aberrant spindle checkpoints. In addition, advanced maternal age is widely acknowledged as the single most important risk factor for the transmission of abnormal chromosome complements to pre-implantation embryos. Emphasis has traditionally been placed on the study of meiotic chromosome segregation in mice and humans. While few studies have focused on other species, early embryonic loss in mares older than 18 years of age is known to reach 30% or higher and has considerable economical impact on the equine industry in the US. Yet, little is known regarding the pathways and molecular mechanisms involved in oocyte meiosis in the horse. Therefore, the objective of this study was to validate specific marker proteins for high-resolution microscopic analyses of meiotic chromosomes in this species.

Using transvaginal ultrasound-guided follicle aspiration (TVA) following gonadotropin treatment, oocytes were retrieved and fixed for immunochemistry. Laser-scanning confocal microscopy was used to perform 3D reconstructions and digital rotations of the equine metaphase plate to determine chromosome alignment and spindle morphology in whole-mount oocytes. Some oocytes were in vitro matured to the metaphase-II stage for numerical and structural analysis of surface spread chromosomes.

Antibodies detecting covalent modifications at core histone tails, such as tri-methylation of lysine 9 of histone 3 (H3K9me3), and spindle microtubule β-tubulin revealed conserved localization patterns with high specificity. In addition, specific anti-CREST antibodies recognizing spindle attachment points enabled an unprecedented level of resolution for fine-structural and quantitative chromosome analysis, as each of the 32 chromosomes in a haploid metaphase-II oocyte spread was labeled by two distinct CREST signals.

Our results demonstrate that inter-species conservation of chromosomal marker proteins in conjunction with high-resolution confocal microscopy is a feasible approach for the study of equine meiosis and may provide insight into the incidence of aneuploidy in older mares.

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A Respiratory Syncytial Virus (RSV) Nanocapsule Vaccine Containing a G Protein Peptide Payload Induces Robust B and T cell Immunity and Protects from Infection and Disease. 

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Respiratory syncytial virus (RSV) is the most important cause of serious lower respiratory tract illness in infants and the elderly worldwide. Currently, no safe and efficacious RSV vaccine exists. Progress in our understanding of RSV infection has shown that RSV G protein contains a CX3C chemokine motif (aa 182-186) that modifies the activity of fractalkine (CX3CL1) affecting immunity and disease pathogenesis. Recent evidence shows that immunization of mice with the central conserved region of the RSV G protein generates antibodies that inhibit G protein CX3C-CX3CR1 interaction and reduce disease pathogenesis. In this study, mice were vaccinated with nanocapsule vaccines produced by layer-by-layer assembly of oppositely charged polypeptides that are terminally loaded with one of the three polypeptides comprising of the CX3C motif from the RSV G protein (GA2, GB1 or GCH17) plus or minus an immunodominant RSV M2 epitope. The findings show that mice immunized with the G protein nanocapsule vaccines produced neutralizing antibody responses associated with reduced pulmonary virus replication following RSV A2 challenge. Additionally, ELISPOT analysis showed that vaccinated mice had significantly increased levels of RSV G-specific IL-4 and IFN-γ secreting cells compared to unvaccinated mice following RSV A2 challenge. Interestingly, the results also revealed that nanocapsule vaccination induced increased levels of RSV M2-specific IL-4 and IFN-γ secreting cells, and by M2-specific H-2Kd-tetramer staining, indicated that vaccinees had increase in the number of M2-specific CD8 T cells in the lungs compared to the unvaccinated group. Importantly, vaccination was not associated with increased pulmonary neutrophil or eosinophil populations following RSV challenge showing that demonstrate that vaccination with the RSV G nanocapsule vaccines is robust, safe and effective.
Infection of surgical wounds remains a problem in veterinary surgery, and the surgical team is a potential source of contamination. The purpose of the study reported here is to determine the prevalence and risk factors of intraoperative glove perforation in small animal veterinary medicine, and to determine the correlation between glove perforation and bacterial glove contamination as well as between bacterial glove contamination and post-operative infection.

During the study period, all gloves from the intraoperative team were collected and evaluated for glove punctures using a water pressure test. The outer surface of each glove of the primary surgeon was cultured at the end of the surgery before the gloves were removed using a modified Gaschen bag technique. All gloves cultured were from non-contaminated surgeries. Other variables recorded were: the type of procedure, duration of the surgery procedure, and the years of experience of primary surgeon. All bacteria recovered from the gloves were characterized. Cases were followed for 2 weeks after surgery to determine the presence or absence of post-operative incisional infection.

A total of 562 gloves were tested for perforations, the percentage of perforated gloves was 10.3%, and in only 18% of cases the staff was aware of the presence of perforations. Gloves from the primary surgeon were cultured during 21 procedures both in soft tissue and orthopedic surgeries. In these cases we observed; No contamination in 38% of the cases, minimally detectable contamination below our capacity to quantitate in 23.8% of the cases, contamination with 5-100 colonies in 19% of the cases, and contamination with more than 100 colonies in 19% of the cases. 19% of the recovered bacteria showed little evidence of pathogenic potential, and 81% showed high pathogenic potential based on the reactions on colonies on blood agar and EMB agar. The most common bacterial isolate were gram positive cocci and gram negative rods. Two patients developed an incisional infections, 2 patients died following surgery, one patient developed an incisional seroma. The rest of the patients had no complications, 35% of which were given antibiotics post-operatively. No significant correlation was found between glove perforation and bacterial glove contamination and between bacterial glove contamination and post-operative infection.

The vast majority of glove perforations occur without staff awareness. A larger study is needed to confirm the true correlation between glove punctures and bacterial glove contamination and between bacterial glove contamination and post-operative infection.
Assessment of diabetic control in veterinary patients relies on several determinants including owner perception of their pet’s thirst, appetite, and urination habits; as well as objective measures like body condition scoring and serial blood glucose curves. Continuous interstitial glucose monitoring (CIGM) has been validated to accurately reflect veterinary patient blood glucose levels. This ongoing study compares treatment recommendations based on patient clinical signs and interpretation of specific components of CIGM curves. Our hypothesis is that clinical signs and the first 24 hours of the CIGM curve are less accurate in predicting diabetic control than the last 24+ hours of the same CIGM curve.

Medical records were searched for diabetic patients who had CIGM curves performed at UGA (n = 232). Data transcribed into a standardized form included clinical signs (% change in weight, food/water intake, urination habits, lethargy/tremors/seizure activity), insulin type with time administered, and a component of the CIGM curve (first 24 hours, last 24+ hours, and the entire 72-hour curve). Two board-certified internists, blinded to patient identities, assessed if diabetes was controlled based only on clinical signs. Anonymous CIGM curve components were evaluated by the same blinded-internists who elected to increase, decrease, or maintain current insulin dose, or change insulin type based solely on curve interpretation.

Intra-clinician agreement between interpretation of clinical signs and the first 24, last 24+, or entire 72 hours of a CIGM curve were evaluated. Inter-clinician agreement was calculated for decisions based on clinical signs alone, and for each component of the CIGM curves. Current analysis suggests poor correlation between interpretation of clinical signs and CIGM curve to assess diabetic control, both within and between clinicians. Individual clinician treatment recommendations also varied depending on the section of CIGM curve evaluated (first 24 hours vs. last 24+ hours).
CONFOUNDING FACTORS IN ALGOMETRIC ASSESSMENT OF MECHANICAL THRESHOLDS IN NORMAL DOGS

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Validated objective or semi-objective measures of tissue sensitivity are needed for clinical pain research. Algometry is a non-invasive method of measuring tissue sensitivity. The purpose of this study was to evaluate algometric readings in normal dogs in a design that would also look at possible confounding influences.

Nineteen skeletally-mature orthopedically and neurologically normal retriever or retriever mix dogs were recruited. Fourteen common surgical sites were selected for algometric pressure testing. Threshold response was defined as a higher center recognition of the stimulus, and recorded in pounds of force. Sites were tested in the same order, and the testing sequence repeated 3 times for each side of the dog. The patients were tested in the morning and evening of a single day; this was repeated 10-14 days later, allowing 4 separate data collections for each patient. Data were analyzed using ANOVA or ANCOVA.

When all the data was included in the analysis, dog (p<0.0001), order (p<0.0001), site (p<0.0001), site order (p=0.0217), time (p<0.0001), day (p<0.0001) and rep (p<0.0001) all significantly affected the algometer readings. When just the first reading for each site was included in the analysis, dog (p<0.0001), site (p<0.0001) and sex (p<0.0001) all significantly affected algometer readings. The data suggest that learning occurred over repeated collection time points, with dogs anticipating the algometer stimulus and reacting at lower thresholds. Therefore, establishment of normal algometric measurement for dogs using this methodology was not possible. Algometry will only be useful if careful experimental design can negate these potential confounding influences.
PLASMA AND PULMONARY DISPOSITION OF CEFTIOFUR AND ITS METABOLITES AFTER INTRAMUSCULAR ADMINISTRATION OF CEFTIOFUR CRYSTALLINE FREE ACID TO WEANLING FOALS

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The objectives of this study were to determine the plasma and pulmonary disposition of ceftiofur crystalline free acid (CCFA) in weanling foals and to compare the plasma pharmacokinetic profile of weanling foals to that of adult horses.

A single dose of CCFA was administered intramuscularly (IM) to 6 weanling foals and 6 adult horses at a dose of 6.6 mg/kg of body weight. Concentrations of desfuroylceftiofur acetamide (DCA) were determined in the plasma of all animals, and in pulmonary epithelial lining fluid (PELF) and bronchoalveolar lavage (BAL) cells of foals.

After IM administration to foals, median time to maximum plasma and PELF concentrations was 24 h (12-48 h). Mean (± SD) peak DCA concentration in plasma (1.44 ± 0.46 µg/mL) was significantly higher than that in PELF (0.46 ± 0.03 µg/mL) and BAL cells (0.024 ± 0.011 µg/mL). Time above the therapeutic target of 0.2 µg/mL was significantly longer in plasma (185 ± 20 h) than in PELF (107 ± 31 h). The concentration of DCA in BAL cells did not reach the therapeutic concentrations. Adult horses had significantly lower peak plasma concentrations and area under the curve compared to foals.

Based on the results of this study, CCFA administered IM at 6.6 mg/kg in weanling foals provided plasma and PELF concentrations above the therapeutic target of 0.2 µg/mL for at least four days and would be expected to be an effective treatment for pneumonia caused by S. zooepidemicus at doses similar to the adult label.
A COMPARISON OF CARDIOPULMONARY AND ANESTHETIC EFFECTS OF AN INDUCTION DOSE OF ALFAXALONE OR PROPOFOL IN DOGS

Jill Maney, Molly Shepard, Christina Braun, Jeannette Cremer, Erik Hofmeister

The purpose of the study was to compare the physiological parameters, arterial blood gas values, induction quality, and recovery quality after IV injection of alfaxalone or propofol in dogs.

The study was designed as a prospective, randomized, blinded crossover using eight random-source adult female mixed-breed dogs.

Dogs were assigned to receive either propofol 8 mg kg\(^{-1}\) or alfaxalone 4 mg kg\(^{-1}\) to effect, then received the alternative drug after a 6-day washout. Temperature, pulse rate, respiratory rate, direct blood pressure, and arterial blood gases were measured before induction, immediately post-intubation, and at 5 minute intervals until extubation. Quality of induction, recovery, and ataxia were scored by a single blinded investigator. Duration of anesthesia and recovery, and adverse events were recorded.

The mean doses required for induction were 2.6 ± 0.4 mg kg\(^{-1}\) alfaxalone and 5.2 ± 0.8 mg kg\(^{-1}\) propofol. After alfaxalone, temperature, respiration, and pH were significantly lower, and P\(_{aCO2}\) significantly higher at post-induction compared to baseline (p<0.03). After propofol pH, P\(_{aO2}\), and S\(_{aO2}\) were significantly lower, and P\(_{aCO2}\), HCO\(_3\)-, and P\(_{a-aO2}\) gradient significantly higher immediately post-induction compared to baseline (p<0.03). Immediate post-intubation and 5-minute physiologic and blood gas values were not significantly different between alfaxalone and propofol. Alfaxalone resulted in a significantly longer time to achieve sternal recumbency (p=0.0003) and standing (p=0.0004) compared to propofol. Subjective scores for induction, recovery, and ataxia were not significantly different between treatments; however, dogs undergoing alfaxalone anesthesia were more likely to have >1 adverse event (p=0.041). There were no serious adverse events in either treatment.

There were no clinically significant differences in cardiopulmonary effects between propofol and alfaxalone. A single bolus of propofol resulted in shorter recovery times and fewer adverse events than a single bolus of alfaxalone.
Class II major histocompatibility complex (MHC) is an independent predictor of outcome in human B-cell lymphoma. We assessed class II expression together with other markers for their impact on prognosis in canine B-cell lymphoma.

We hypothesized that low class II MHC expression, large cell size, and expression of CD34 will predict a poorer outcome in canine B-cell lymphoma. Expression of CD5 and CD21 on tumor cells also may be associated with outcome.

One hundred and sixty dogs with cytologically confirmed lymphoma were enrolled in this study. Patient signalment, treatment type, and flow cytometry characteristics were analyzed for their influence on outcome. A multivariable predictive model of survival was generated using 2/3 of the patients and validated on the remaining 1/3 of the dataset.

Class II MHC expression had a negative association with mortality and relapse. Treatment type also influenced relapse and mortality, whereas cell size and patient age was only associated with mortality. CD34, CD21, and CD5 expression was not associated with disease outcome. The constructed model performed variably in predicting the validation group's outcome at the 6-month time point.

Low levels of class II MHC expression on B-cell lymphoma predict a poor outcome, as in human B-cell lymphoma. This finding has implications for the use of dogs to model human lymphomas. Class II expression, cell size, treatment, and age can be combined to predict mortality with a high level of specificity.
LAPAROSCOPIC ASSISTED OVARIECTOMY USING A BIPOLAR VESSEL SEALING DEVICE

Sherisse Sakals, Clarence Rawlings, Jamie Laity, Erik Hofmeister, MaryAnn Radlinsky

The technique and results of a laparoscopic assisted approach to ovariectomy in cats using a bipolar vessel sealing device are reported. Our hypotheses were 1) laparoscopic assisted ovariectomy can be achieved using a bipolar vessel sealing device, 2) laparoscopic assisted ovariectomy can be performed with equivalent post-operative comfort when compared to traditional ovariohysterectomy.

Thirty healthy female cats were randomly assigned to one of three groups: laparoscopic assisted ovariectomy using a bipolar vessel sealing device (Group A), laparoscopic assisted ovariectomy using ligation (Group B), or ovariohysterectomy (Group C). Data collected included surgery time, interactive visual analogue scale (IVAS) pain scores, and blood glucose and cortisol levels.

For Group A, an abdominal port was established for the 2.7 degree endoscope and each ovary located. Hemostats were advanced through the lateral body wall to grasp and exteriorize each ovary for removal using the bipolar vessel sealing device. For Group B, the procedure was the same with the exception that ovarian pedicle hemostasis was achieved using ligation. Ovariohysterectomy was performed using a standard open technique.

Successful ovariectomy was performed using the techniques described. There was no significant difference in the IVAS data or the blood parameters between any of the three groups. Surgical time was shortest for Group C and there was no difference in time between Groups A and B.

Laparoscopic assisted ovariectomy using either a bipolar vessel sealing device or suture ligation can be used to sterilize female cats with the same level of post-operative comfort for the patient as traditional ovariohysterectomy.

A. Wang, J.R. Smith

This retrospective evaluated the clinical use and adverse effects of mycophenolate mofetil (MMF) for treating canine primary immune-mediated hemolytic anemia (IMHA).

UGA medical records (2007-2011) were searched for the coded diagnosis of IMHA (n=93). Inclusion criteria included PCV<35%, with positive slide agglutination test, spherocytosis, evidence of hemolysis and/or positive Coombs’ test. Exclusion criteria included IMHA secondary to infectious causes, drug toxicity, or neoplasia (n=29). Data collected included signalment, initial vital parameters, body condition score, clinicopathologic data, imaging results, medications administered, duration of hospitalization, short term survival times, and adverse effects.

Of 64 primary IMHA cases, 57 received steroids and a secondary immunosuppressive: 31 mycophenolate, 15 cyclosporine, 6 azathioprine, 1 human immunoglobulin. Seven dogs received three immunosuppressives, 2 patients received only steroids; 2 patients were immediately euthanized. There was no significant difference between signalment or clinicopathologic data in dogs given MMF or other secondary immunosuppressives.

Data are presented as mean ± SD for dogs receiving MMF. Initial PCV was 17 ± 5.2%. Initial MMF dose was 20.5 ± 5.8 mg/kg PO or IV divided twice daily. Diarrhea was the only adverse effect noted (n=5), although one dog was presented with diarrhea. Number of transfusions received was 1.9 ± 1.6; hospitalization duration was 5.7 ± 4.5 days. Twenty-four dogs (77.4%) survived to discharge, 2 were lost to follow-up, and 18 dogs were alive at study conclusion. Mean survival time was 13 days in non-survivors.

In conclusion, oral and IV administration of mycophenolate mofetil is a safe and effective additional immunosuppressive for treating primary canine IMHA.
Examining the Biological Function of Resuscitation Promoting Factor B (RpfB) -- in Mycobacterium shottsii and Mycobacterium pseudoshottsii

Tuhina Gupta, Jonathan Noel, Russell Karls, and Frederick Quinn

Abstract

Resuscitation promoting factors are thought to be important for the re-establishment of late stationary phase and potentially latent cultures of various Mycobacterium species, including M. tuberculosis. In the M. tuberculosis genome, five genes encoding resuscitation factors-rpfA, rpfB, rpfC, rpfD and rpfE have been identified thus far. Studies by other investigators have shown that of these five known resuscitation factors, RpfB is essential for long term survival and re-growth of M. tuberculosis under nutrient depletion and other conditions of severe stress. Mycobacterium shottsii and M. pseudoshottsii have been identified as agents of recent outbreaks of mycobacterioses in Chesapeake Bay striped bass. Pathogenic mycobacteria possess notoriously slow replication rates. Unfortunately, these newly-identified species replicate more slowly than all but one other pathogenic Mycobacterium species, thus delaying diagnostic results and therapeutic interventions. Our goals were to determine if resuscitation-promoting factor B (Rpf B) was produced by M. shottsii and M. pseudoshottsii and determine if this factor when added to culture medium could enhance replication rates of these species. We successfully PCR-amplified rpfB from both M. shottsii and M. pseudoshottsii using primers designed to detect an rpfB analog in the closely-related species, M. ulcerans. This sequence is also nearly identical to rpfB in M. tuberculosis. Thus, various concentrations of spent M. shottsii and M. pseudoshottsii media from stationary phase cultures (O.D 600 nm = 1.0), were used to compare the growth of very old (eight- twelve months) cultures of these species. Changes in mycobacterial culture were measured by regular optical density measurement at 600 nm. It was observed that adding spent media to new culture had growth-enhancing ability. Interestingly, there may also be a role for these factors in disease since antibodies generated against purified M. tuberculosis RpfB could detect the presence of the analogous M. shottsii and M. pseudoshottsii proteins in spleens of infected fish.
ALLERGY SKIN TEST RESULTS IN 269 ATOPIC DOGS USING 59 ALLERGENS IN TWO DIFFERENT TEST CONCENTRATIONS

Patrick Hensel

Intradermal testing (IDT) is considered the most effective test to identify offending allergens in dogs suffering from atopic dermatitis (AD). However, occurrence of false positive and false negative reactions has been reported in normal non-allergic and allergic dogs. Recent studies have shown that higher intradermal test concentrations (ITCs) can be used without triggering false-positive irritant reactions and side effects, while dust mites should be tested with lower ITCs. In this study, these ITCs are referred to as the adjusted ITC and were compared to the ITCs which are used by most dermatologists which are referred to as the standard ITCs. The aim of this study was to determine which allergens atopic dogs react to the most, and to assess the difference between the standard and adjusted ITCs on dogs with atopic dermatitis.

IDT, using 59 different allergens, was performed in 269 dogs with a preliminary diagnosis of AD. The standard ITC for plant, mold and insect allergens was 1000 PNU/ml, 500 PNU/ml for animal hair and dander, and 250-500 PNU/ml for dust mites. The adjusted ITC for plant, mold and insect allergens was 1250-8000 PNU/ml, 1250 PNU/ml for animal hair and dander, and 50-200 PNU/ml for dust mites. Results of the adjusted ITC and standard ITC were compared by McNemar’s test and all hypothesis tests were 2-sided and the significance level was α=0.05.

The dog population consisted of 57 different pure bred and mix breed dogs, with Golden and Labrador Retriever to be the most predominant breeds. Median age was 4.2 (minimum to maximum value range, 5 months to 12.25 years) years. The IDT in 64 dogs was negative. The lowest number of reaction was seen for duck and goose feathers, while the highest number of reactions was seen to Dermatophagoides farinae. Significantly more positive reactions were observed with adjusted ITC vs. standard ITC for Cocklebur (p=0.0082), Red Maple (p=0.0196), and Alternaria tenuis (p=0.0455). Significantly more negative reactions were observed with adjusted ITC vs. standard ITC for Dermatophagoides farinae (p<0.0001), Dermatophagoides pteronyssinus (p<0.0001), and Tyrophagusputrescentiae (p=0.006). For most other allergens an increased number of positive reactions were observed with the adjusted ITC, but were not statistically significant. The findings of this study suggest that the use of adjusted ITC for dust mites may result in less false positive reactions, while for the other allergens the use of higher ITC may yield more positive reactions, which may be missed with the standard ITC. Despite using adjusted ITC, 64 atopic dogs had a negative IDT. Reasons could be that the dogs were allergic to allergens which have not been included in the IDT or they may suffer from atypical AD. Further studies will be needed to assess whether desensitization, based on the IDT results using the adjusted ITC results, can result in increased success rate of desensitization.
PHARMACODYNAMICS OF ALFAXALONE AFTER SINGLE-DOSE INTRAMUSCULAR ADMINISTRATION IN RED EARED SLIDERS (TRACHEMYS SCRIPTA ELEGANS): A COMPARISON OF TWO DIFFERENT DOSES AT TWO DIFFERENT AMBIENT TEMPERATURES.


Published anesthetic effects of alfaxalone in chelonians are currently limited to anecdotal and case reports. This blinded crossover experimental study compares the pharmacodynamics of two different intramuscular alfaxalone doses administered to red eared slider turtles in two different ambient temperatures. Following 2-week acclimation at 72-77 degrees F, nine adult female sliders were randomly assigned to two groups: W10 (warm 10mg/kg) and W20 (warm 20mg/kg). A blinded observer evaluated heart rate, palpebral and corneal reflex, muscle relaxation and ease of handling (described via 3-point scale), and sensitivity to a toe-pinch stimulus at baseline (pre-injection), and at 5, 12, 20, 30, 45, 60, and 120 minutes post-injection. Turtles then acclimated to 65-68 degrees F for 60 days, alfaxalone dose was again randomized, yielding the treatment groups C10 (cold 10mg/kg) and C20 (cold 20mg/kg), and the experiment repeated. By the end of the study, each turtle received each dose in each temperature condition, with a minimum 7-day washout period. According to repeated-measures ANOVA comparing area under the curve for each parameter in each treatment condition: C10 and C20 turtles had significantly lower heart rates than W10 and W20 turtles. C10 turtles were significantly more relaxed and easier to handle than W10 turtles. W20 turtles were significantly more relaxed and easier to handle than W10 turtles. No significant differences in palpebral reflex, corneal reflex or response to toe-pinch were found between treatment groups. Anesthetic duration was significantly longer in C20 turtles compared to C10 turtles. Anesthetic duration was also significantly longer in W20, C20 and C10 turtles compared to W10 turtles. Significance was set at p≤0.05.

The behavioral responses we observed in this study suggest that IM alfaxalone is a viable injectable agent for turtles undergoing minimally-invasive procedures (e.g. IV catheterization, minor laceration repair) but should not be used as a sole anesthetic in animals undergoing potentially painful procedures.
PERICENTRIN REGULATES MEIOTIC SPINDLE STABILITY IN OOCYTES

Wei Ma and Maria M. Viveiros

An abnormal chromosome number (aneuploidy) in developing embryos is the leading genetic cause of congenital birth defects and pregnancy loss. The majority of aneuploidies are attributed to errors in chromosome segregation that occur during meiotic division in oocytes. Accurate chromosome segregation is critically dependent on assembly of the microtubule spindle apparatus as well as the establishment of stable chromosome-microtubule attachments. Importantly, spindle formation in oocytes differs from mitotic cells and is regulated by unique microtubule organizing centers (MTOCs) that lack centrioles. Yet, despite its importance, the molecular composition of MTOCs and control of spindle assembly in mammalian oocytes is poorly understood.

To assess MTOC function in oocytes, we tested the role of a key MTOC-associated protein, pericentrin. In somatic cells, pericentrin reportedly functions as a scaffolding protein that integrates an array of regulatory factors at MTOCs including γ-tubulin, which is essential for microtubule nucleation. Whether pericentrin function is conserved in oocytes is not known. Using immunofluorescence analysis, pericentrin was localized specifically to MTOCs in prophase-I arrested mouse oocytes recovered from pre-ovulatory ovarian follicles, as well as during meiotic maturation. To evaluate function, pcnt transcripts were knocked down using specific siRNAs. Both, western blot and immunofluorescence analysis confirmed efficient protein depletion in the majority (>80%) of oocytes injected with pcnt siRNAs. Labeling of DNA and spindle microtubules with DAPI and anti-acetylated tubulin antibodies, respectively, revealed highly disrupted meiotic spindle organization and chromosome attachment in oocytes with little or no pericentrin. Most oocytes (~70%) from the pcnt siRNA group contained disorganized spindle structures with misaligned, or lagging, chromosomes. Moreover, the localization γ-tubulin to MTOCs was disrupted following pericentrin depletion. These data indicate that pericentrin is essential for meiotic spindle organization and stability in mammalian oocytes. In subsequent studies we propose to generate an oocyte-conditional Pcnt knockdown mouse model using an established transgenic RNAi approach. This will provide a unique genetic model to directly test MTOC-mediated microtubule nucleation and organization in mammalian oocytes.
Epidemiological studies of hyperthyroid cats have implicated consistent risk factors for the disease to include age and exposure to canned cat food. Flavonoids which are soy proteins and known endocrine disruptors have been found in high concentrations in cat food. As cats are obligate carnivores, such plant proteins may represent a novel food source, not encountered in the wild. The aim of this study was to determine the effects of flavonoids on thyroid cell activation in culture. A previously characterized feline hyperthyroid cell line, PetCat2, was used for this study. Cells were passed onto coverslips and starved for 6 days to reduce cell activation to basal levels. One hundred μM thyroid stimulating hormone (TSH), 1nM-10 mM of flavonoid compounds quercetin, myrecetin, biochanan A, genistein or vehicle was added along with bromodeoxyuridine (BrdU). Cells were incubated for 48 hours, and positive fluorescence was assessed using epifluorescence microscopy. Mitogenesis and thyroglobulin (Tg) syntheses were assessed by quantifying the cells positively stained with BrdU or anti-thyroglobulin antibody as a percentage of the total cells present in the same microscopic field. Thyroglobulin synthesis was assessed using anti-thyroglobulin antibodies. Basal mitogenesis and Tg synthesis of the starved PetCat 2 cells in culture were 5 +/- 2% and 9 +/- 3%, respectively. Upon the addition of TSH, BrdU and Tg positive cells increased significantly (p=0.01) to 45 +/-10% and 50 +/-9%, respectively. Quercetin, myrecetin, and genistein caused an increase in BrdU uptake in the cells to 77 +/-18%, 85 +/-9%, and 65 +/-13%, respectively. These were significantly higher than stimulation by TSH (p <0.001). There was no significant stimulation of mitogenesis by Biochanin A. Thyroglobulin synthesis was stimulated by all the flavonoids to a significant level over background (quercetin 85 +/-10%, myrecetin 89 +/- 8%, biochanan A 65 +/- 5%, and genistein 67 +/- 14%). Stimulation of mitogenesis and Tg synthesis by the flavonoids was concentration-dependent with the greatest effect seen at 10-100 μM. These results demonstrate that flavonoids, can activate feline hyperthyroid cells in culture.
The Basic Effectives of Dextran on Human Monocyte

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Abstract

HIV/AIDS is one of the worst health problems in the world. The goals of many HIV research programmes are associated with HIV vaccines. Currently, there is no effective vaccine against HIV in clinic, but consistently studies of HIV vaccine are not stopped. Seeking a novel strategy to make HIV vaccine is progress such as polysaccharide-conjugated vaccine. In this study, high molecular weight dextran, one of polysaccharide, was used to stimulate human macrophage-like U937 cells to study the basic effectives on response to dextran treatment and on preliminary possibly related pathways. Our data demonstrated that 40 µg/ml of dextran was not induced the significant alteration of important gene expressions but decreased the gene expressions of CXCR4 and CCR5 mediated with mitochondria. By the way, interesting observation in the investigation of the effect of the dextran on cell viability was found that PI13 K and NFκB pathway played a very important role in the cell viability and ROS induced by mitochondria had partially influenced the cell viability. Regarding Toll-like receptors, dextran stimulated TLR1 increase, TLR9 decrease via PI13K pathway; however the alterations were restored by HIV-1 gp 120. Our data also showed that PI13K, mitochondrial ETC and NFκB pathways plays very key roles in regulating perforin and CD260 and dextran was not induce ROS generation. In conclusion, dextran is one of good conjugate polysaccharide to develop HIV vaccine.