

TARGETING SPECIFIC DOPAMINERGIC PATHWAYS WITH CRE-DEPENDENT DREADDS

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Schizophrenia remains to be a poorly understood and complicated disease that alters the CNS by affecting the mesocortical, mesolimbic and nigrostriatal dopaminergic pathways. This lack of an understanding of the disease leads to a challenge in treating the extrapyramidal symptoms caused by the conventional antipsychotic drugs. There is a need to develop an antipsychotic treatment that can target the excess dopamine neurotransmission in the mesoaccumbens pathway, enhance the mesocortical pathway and neglect to affect the nigrostriatal pathway. In order to develop this type of beneficial treatment, we need to understand how gene expression is impacted in dopamine neurons from the use of antipsychotics. Therefore, our goal was to investigate the feasibility of using a combination of a Cre-dependent adeno-associated viral vectors expressing DREADDS (Designer Receptors Exclusively Activated by Designer Drugs) in combination with the retrogradely transported herpes simplex viral vector expressing Cre into either the prefrontal cortex, nucleus accumbens, or substantia nigra to target these distinct pathways to inhibit or stimulate them. Our data indicates that AAV can infected dopamine neurons and that HSV is retrogradely transported in neurons. Additionally, we found that DREADDS can be expressed in specific dopamine neurons, based on efferent innervation, can be achieved by using AAV and HSV. One caveat to this approach is that only a small subset of dopamine neurons were labeled by this method. Future studies will investigate molecular changes that result from altering activation of these dopamine neuron populations in ways that mimic what occurs in patients with schizophrenia.

CHARACTERIZATION OF EXTENDED SPECTRUM CEPHALOSPORIN RESISTANT *ENTEROBACTERIACEAE* ISOLATED FROM COMPANION ANIMALS

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Extended spectrum cephalosporin resistant *Enterobacteriaceae* (ESCRE) is a growing problem in public health, but little is known about ESCRE in companion animals. 50 *Klebsiella pneumoniae*, 8 *Klebsiella oxytoca*, and 35 *Escherichia coli* were collected from the veterinary diagnostic laboratory at Mississippi State University from 2015 to 2017. Phenotypes of ESCRE and genotypes of extended spectrum β -lactamase (ESBL) were analyzed using antibiotic sensitivity test and multiplex PCR, respectively. 19 *K. pneumoniae* isolates (38%) showed ESCRE resistance to multiple cephalosporin classes including cefazolin (n=19, 38%), cefoxitin (n=7, 14%), cefpodoxime (n=17, 34%) and cephalothin (n=18, 36%) and isolates carried multiple ESBL genes (TEM n=21, 42%, OXA-1 n=15, 30%, CTX-M group 1 n=11, 22%, and CIT n=4, 8%). 2 *K. oxytoca* isolates were resistant only to cefazolin with no ESBL genes detected. 8 *E. coli* isolates (22.6%) were ESCRE showing resistance to cefazolin (n=6, 17.1%), cefoxitin (n=3, 8.5%), cefpodoxime (n=6, 17.1%) and cephalothin (n=8, 22.6%) and also carried multiple ESBL genes (TEM n=5, 14.3%, OXA-1 n=2, 5.7%, CTX-M group 1 n=3, 8.6%, and CIT n=7, 20%). The resistance to 1st and 3rd generations of cephalosporin by ESCRE was correlated with the heavy use of cefazolin/cephalexin in veterinary hospitals. The ESBL genes found are encoded in transferrable plasmids, which also poses a potential public health risk to the increased emergence of ESCRE. Considering the close interaction between humans and companion animals, this study suggests a need for nationwide investigation of ESCRE in veterinary medicine and establishing an antibiotic stewardship program with One Health concept to prevent ESCRE threats in public health.

GLUCOCORTICOID RECEPTOR EXPRESSION IS DECREASED WITH NUCLEAR LOCALIZATION IN AIRWAYS OF PASTURE ASTHMA HORSES

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Severe human asthma is defined as poorly responsive to inhaled glucocorticoids. We investigate a spontaneous animal asthma model, equine pasture asthma (EPA), affecting grazing horses during summers in the southeastern US. EPA recapitulates characteristics of severe human asthma including neutrophilic airway inflammation, and hyper-responsiveness to methacholine bronchoprovocation diagnostic of severe asthma (<1mg/ml). Our analysis of the EPA lung transcriptome identified decreased glucocorticoid receptor (GCR) expression. We hypothesize that GCR protein expression is decreased in bronchioles of horses with pasture asthma. Based upon predicted conservation of epitopes between equine and human GCR (100% nucleotide and amino acid homology), antibodies to human GCR were employed to characterize GCR protein expression in lung tissue from horses during clinical EPA exacerbation. Compared to non-diseased control horses, distinct decreases in GCR expression were identified in bronchiolar epithelium from EPA horses. Moreover, nuclear staining predominated in bronchiolar epithelium of EPA horses. In contrast, both cytoplasmic and nuclear staining were evident in bronchiolar epithelium from control horses. We conclude that GCR protein expression is decreased and its intracellular localization is shifted primarily to the nucleus in airways of EPA horses. These findings are congruent with descriptions of nuclear GCR localization and decreased GCR binding affinity in Type I steroid resistant human asthmatics, and align to anecdotal descriptions of EPA as poorly responsive to glucocorticoid administration without removal from inciting environmental factors.

EFFECT OF THE MARIJUANA COMPOUND CANNABIDIOL (CBD) IN MILD AND MODERATE AUTOIMMUNE DISEASE

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Autoimmune diseases in veterinary medicine can be difficult to treat. With increased use of marijuana compounds in humans, there is interest to understand the mechanisms of these compounds to treat either human or animal autoimmune diseases. We established the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis to examine the effects of CBD as a possible treatment. We induced mild and moderate EAE disease in mice then treated them for five days with 75 mg/kg CBD by oral gavage. We hypothesized CBD will attenuate EAE by preferentially suppressing neuroinflammation with little effect on the peripheral immune response. We examined immune function and neuroinflammation at days 3, 10, and 18 after disease initiation. CBD attenuated clinical disease as shown by clinical score and onset of disease. Assessment of pro-inflammatory cytokine production in the spleen showed that cytokine production was maximal in moderate and mild disease at day 10. CBD significantly inhibited IFN- γ in both CD4+ and CD8+ T cells in moderate disease but not mild disease. IL-17A production was not significantly altered by disease or CBD. The mechanism for immune suppression was not dependent on regulatory cells. Neuroinflammation as assessed by T cell infiltration into the brain demonstrated robust T cell staining in moderate disease which was decreased by CBD at day 18. The results suggest that the mechanism by which CBD decreases disease involves early suppression of IFN- γ production in the periphery and later suppression of neuroinflammation. The significance of this work is that CBD could be beneficial for veterinary autoimmune diseases.

EFFECTS OF A *LISTERIA MONOCYTOGENES* PHOSPHODIESTERASE, PDEE, ON PHENOTYPES CONTROLLED BY CYCLIC DINUCLEOTIDES

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Listeria monocytogenes is an important foodborne pathogen that causes infection most commonly in pregnant women, newborns, elderly, and immunocompromised people. It is capable of replication inside host macrophages and enterocytes. Previously we discovered a listerial phosphodiesterase (PdeE) that is important for intracellular replication and virulence, and we determined that the enzyme hydrolyzes cyclic-di-AMP and cyclic-di-GMP. These cyclic dinucleotides are important signaling molecules that regulate listerial adaptation to different environments, including growth in the host and in biofilms. Listerial strains that have high levels of cyclic-di-GMP have distinct phenotypes, including binding Congo red and decreased motility. Similarly, listerial strains with high cyclic-di-AMP have slower growth. We hypothesize that overexpression of PdeE in *L. monocytogenes* strains that have high levels of cyclic-di-AMP or cyclic-di-GMP will reverse these phenotypes due to hydrolysis of these cyclic dinucleotides. To test this hypothesis, we overexpressed the *pdeE* gene from a plasmid and inserted it into *L. monocytogenes* mutants with high cyclic-di-AMP or cyclic-di-GMP. To confirm overexpression of PdeE from the plasmid, RNA was extracted from each strain and used for quantitative PCR (qPCR). Colony size, motility, and ability to bind Congo red was analyzed to determine whether overexpression of PdeE would reverse the phenotypes caused by high cyclic di-AMP and/or cyclic di-GMP. If our hypothesis is correct, this will be the first time an enzyme is found in *L. monocytogenes* that has confirmed hydrolytic activity against both cyclic-di-AMP and cyclic-di-GMP and can control phenotypes affected by both cyclic dinucleotides.

THE EFFECTIVENESS OF THE TRANSCUNEAL EXTRACORPOREAL SHOCKWAVE THERAPY ON EQUINE NAVICULAR SYNDROME

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Equine researchers and veterinarians speculate that approximately 90% of lameness in horses stems from the foot and that navicular syndrome is one the most common causes of forelimb lameness in horses (1). Equine Navicular Syndrome (ENS) is a chronic debilitating disorder involving the equine navicular bone and its surrounding soft tissue structures. The lameness resulting from ENS can be career-ending and is a significant source of loss of use and income in the equine industry. Extracorporeal shockwave therapy (ESWT) is an emerging treatment modality for ENS (2,3). The resultant energy transmitted to underlying tissues results in microdisruption of cells and cell death, creating a controlled inflammatory process that promotes neovascularization and healing (4). Currently, there is no evidence that shock waves can reach the navicular bone, nor is there an established protocol for applying shockwave to the navicular bone. In this study, we evaluated the magnitude of shock waves that reach the navicular bone, and compare the magnitude that reach the navicular bone when the foot was soaked and unsoaked. Piezoelectric sensors were placed over the flexor surface of the navicular bone in order to measure the energy that reaches the bone when shockwave therapy is performed. Then, we measured strain over navicular bone in bisected equine cadaver feet when shock waves were applied to soaked and unsoaked feet. Based on the preliminary data, the shockwave treatment is effective at reaching the navicular bone and soaking the foot allowed a greater amount of shockwave transmission to the navicular bone. Future studies are warranted, but the preliminary results of this study may directly influence clinical protocols for navicular shockwave therapy in veterinary hospitals.

A PILOT STUDY IN THE ESTABLISHMENT OF CANINE UROTHELIAL PRIMARY CELL CULTURES

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Chronic cystitis is a common affliction in both human and veterinary medicine. This research team aims to prove that canine models of disease are appropriate for developing treatments for human chronic cystitis. This study served as the first stepping stone in the model's development, in that this team sought to demonstrate the capability of establishing a primary cell culture for future use in testing potential chronic cystitis treatment options. The approach to this study involved two methods for cell collection – isolating urothelial cells from free catch urine samples and enzymatically digesting the tissue of a bladder harvested from a recently euthanized animal. A total of 33 free-catch urine samples were collected from 11 dogs - 5 castrated males, 3 intact males, 2 spayed females, and 1 intact female. For purposes of refining technique, 1 intact male rat bladder was harvested before harvesting the 2 spayed female canine bladders included in the study. Following cell isolation from both sources, the cells were incubated in 1mL of complete culture media in a cell culture plate. A heterogenous cell population, including epithelial-like cells, was noted on all cultures. The viability of the cell population was assessed using a LIVE/DEAD fluorescence stain. A portion of cells were incubated on a poly-L-lysine coated coverslip as a specimen for scanning electron microscopy to characterize the urothelial cells' morphology. Despite a visibly appreciable increase in the number of cells attached to the culture surface, a complete monolayer was not achieved from the free-catch urine sample cultures. Complete data acquisition from the canine bladder harvests has not yet been achieved due to the timing of the bladder harvest.

ENHANCING ANTIBIOTIC EFFECTIVENESS BY POLOXAMER 407 GEL AGAINST STAPHYLOCOCCUS AUREUS BIOFILMS

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A common clinical problem is chronic wound infections with biofilms of *Staphylococcus aureus*. Systemic treatment against *S. aureus* infection is unsuccessful due to lack of wound vascularization, host tissue death, and biofilm defenses. Effective topical antibiotic regimen could increase success of healing in chronic wounds and accompanying infections. We hypothesize that poloxamer 407 gel, a thermoreversible polymer, can be an effective delivery vehicle by slowly releasing antibiotics to extend pharmacokinetics of an antibiotic dose at the chronic infection site. The objective of this *in vitro* pilot study was to observe varying methods of biofilm treatment and their effectiveness. First, a successful *in vitro* environment of bovine serum coated coverslips submerged at varying levels of serum broth was achieved. Then *S. aureus* biofilm populations were treated with different concentrations of vancomycin to determine susceptibility via colony forming units. The vancomycin concentrations were incorporated into the poloxamer gel as a vehicle for antibiotic delivery. Success of antibiotics with the poloxamer gel was also determined via colony forming units. To better understand viability of the biofilm, plasmids expressing fluorescent GFP and YFP reporter genes were constructed and transformed to the *S. aureus* strain. Biofilm viability can be determined via fluorescent microscopy and confocal laser microscopy with this gene incorporation. Fluorescent microscopy in addition to colony forming units showed promise in quantification of biofilm viability. Further research is needed for successful study of *S. aureus* biofilms and their susceptibility. The long-term outcome is to integrate studied methods into an *in vivo* application.

BLOOD RESPONSE PATTERNS IN JUVENILE KEMP'S RIDLEY SEA TURTLES WITH GRANULOMAS FOLLOWING FISHING HOOK REMOVAL

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Anglers in the Mississippi Sound often unintentionally catch Kemp's ridley sea turtles. Following hook removal, some of these animals develop granulomas where the hook was embedded and removed. To refine diagnostic protocols and examine the turtle's progression of pathophysiologic response to granuloma formation, CBC and blood chemistry results were compared between healthy animals, animals with granuloma and animals deemed releasable after treatment.

Cell counts among groups were compared among using the Kruskal-Wallis and Dunn's tests for multiple comparisons and only differences with $p < .05$ were considered significant. WBC, Heterophils and Urea were higher in animals with granuloma than in healthy and releasable groups. The releasable group had higher Total Protein, Albumin, Globulin, and lower CPK than the other two groups. It also had lower phosphorus and potassium concentration, and higher Na/K ratio than the healthy group. Finally, chloride was highest in the healthy group.

Physiological responses and healing processes in Kemp's ridley sea turtles are not fully understood; this work is a first step towards improving treatment for a common ailment in sea turtles. Our goal is to treat turtles that form granulomas and interpret their blood correctly in order to enhance survival and efficient rehabilitation for preservation of this critically endangered species.

GRAM NEGATIVE ENTERIC BACTERIAL ANTIBIOTIC RESISTANCE IN THE NORMAL FLORA OF SNAKES

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The growing popularity of owning and interacting with exotic animals has increased the chances of humans and animals encountering infectious bacterial agents which can be present in the normal flora. Handling commonly owned reptiles such as snakes and turtles increases the risk of zoonotic disease, especially with antibiotic resistant bacteria. Since the diversity of enteric gram negative bacteria in reptiles was not well known, this study aimed to determine bacterial prevalence, antibiotic resistant bacteria, and specific genes leading to resistance in snake flora. Cloacal swabs were used to acquire samples of enteric bacteria from eleven different snakes. Seven snakes were captive bred and owned, while four were caught in the wild. Enteric bacteria were isolated on specific broth and agar plates and identified using a Sensititre (ThermoScientific) system and analytical profile index. Minimal inhibitory concentration results were also collected. *Salmonella* and *Klebsiella* were isolated from eight individual snakes, *Citrobacter* from seven snakes, and *Morganella* from five snakes. Of the 37 bacterial isolates, 26 (70.3%) were resistant to cefazolin, 24 (64.9%) to cephalothin, and 21 (56.8%) to ticarcillin. Resistance was confirmed by PCR tests that resulted in four isolates presenting a DHA gene, one presenting a CTX2 gene, and one *Providencia rettgeri* isolate that presented with both genes. These genes represent an innate mutation allowing for broad spectrum and AMPc beta-lactamase drug resistance and furthering the infectious potential of these bacteria. Our data confirms the existence of resistance in the normal flora and potential hazards involved with handling these exotic reptiles.

DEVELOPMENT OF AN ELISA TO DETECT BHV-1 SPECIFIC IGA IN BOVINE NASAL SECRETIONS

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Bovine Respiratory Disease (BRD), also known as shipping fever, causes substantial economic losses in stocker and feedlot operations. BRD is a multifactorial disease complex caused by a combination of environmental stress, primary viral infection, and secondary bacterial infection of the lower respiratory tract. Bovine herpesvirus-1 (BHV-1) causes immunosuppression and damage to the upper respiratory tract epithelium, allowing commensal nasopharyngeal bacteria to move into the lower respiratory tract, causing severe bronchopneumonia. Intranasal BHV-1 vaccines are commonly administered to prevent BRD, but it is not clear how stressors such as transport, which sometimes occur before vaccination, affect the mucosal immune response to intranasal vaccines. We developed an enzyme-linked immunosorbent assay (ELISA) to measure IgA specific to BHV-1 in nasal secretions to allow evaluation of the mucosal immune response in future research to determine the impact of management-related stressors on mucosal immunity following vaccination. Development of this indirect ELISA required optimization of concentration of viral antigen, concentration of nasal secretion samples, concentration of secondary anti-IgA antibody, incubation time and temperature, and blocking and wash buffers used. The ELISA successfully identified an increase in specific absorbance signal in nasal secretions collected from calves after intranasal BHV-1 vaccination, as compared to calves sampled before vaccination. However, nasal secretions caused relatively high nonspecific (background) staining; thus work is ongoing to determine the best blocking strategy to decrease nonspecific staining.

PATHOGENESIS OF INFLUENZA D VIRUS AND *MANNHEIMIA HAEMOLYTICA* IN CATTLE

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Bovine respiratory disease (BRD) is one of the most economically significant diseases of cattle and is caused by stress and a primary infection allowing for a severe secondary bacterial infection. Influenza D virus (IDV) is a common microbe identified in cattle diagnosed with BRD and has been significantly associated with some known BRD pathogens. However, IDV's potential role in BRD has not yet been studied. The objective of this study is to evaluate the synergetic pathogenesis in cattle by co-infection of IDV and *Mannheimia haemolytica*, a common bacterium identified in BRD. Sixteen dairy calves were randomly assigned to 4 groups. Groups A and C received IDV (D/Bovine/C00046N/Mississippi/2014) intranasally at 0 days post inoculation (DPI). Groups A and B received *Mannheimia haemolytica* D153 intratracheally at 5 DPI. Group D received neither pathogen. Clinical signs were evaluated and used to calculate clinical scores for each calf. At 10 DPI, calves from groups A, B, and D were euthanized and evaluated for pathologic lesions. All calves in groups A and C seroconverted by 10 DPI, and viral titration suggested these calves shed virus up to 7 DPI. IDV was detected in sera of 3 calves in these groups. Clinical scores rose in both groups after IDV inoculation and returned to normal after 4 and 6 DPI, respectively. After 5 DPI, group B clinical scores rose and remained high for the remainder of the study. There was no significant difference in gross pathology between groups. This data shows no increase in severity of clinical disease caused by *Mannheimia* with prior IDV infection, which suggests that IDV alone does not adequately compromise the host to lead to development of BRD.