College of Veterinary Medicine
Kansas State University
presents

Phi Zeta Research Day

March 5, 2024

Annual Phi Zeta Research Day
March 5, 2024
The Sigma Chapter of Phi Zeta, est. 1969

Schedule of Events

12:00 (noon)	***PLENARY Session***[1]
BI Auditorium	**Welcome and Introduction of Keynote Speaker by Phi Zeta President, Dr. Raghavendra Amachawadi** ***Keynote Speaker*** **Dr. Scott Dee,** Scott Dee, DVM, MS, PhD, DACVM **"Next Generation Biosecurity: The Future of Animal Agriculture."**
1:15 – 2:30 pm	***ORAL Research Presentations***[2]
BI Auditorium	**Applied/Clinical Science Research (Large Animals/Small Animals/Exotics)**
201 Trotter Hall	**Applied/Clinical Science Research (Large animals)**
301 Trotter Hall	**Basic Science Research & Case Reports**
2:30 – 3:30 pm	***Royal Canin POSTER Session***
BI Atrium	**Basic Science Research / Applied/Clinical Science Research / Case Reports**
3:30 – 4:30 pm	***ORAL Research Presentations***[2]
BI Auditorium	**Applied/Clinical Science Research (Large Animals/Small Animals/Exotics)**
201 Trotter Hall	**Applied/Clinical Science Research (Large animals)**
301 Trotter Hall	**Basic Science Research & Case Reports**
4:30-5:30 pm	
BI Atrium	***Reception***
5:30 pm	
BI Auditorium	***AWARDS Ceremony***[3]
	Initiation of New Members to Phi Zeta **Announcement and Presentation of Awards Recognizing Research and Scholarship Accomplishments** **Closing Comments**

[1] The plenary session (welcome and keynote) will be held in the BI Auditorium – Buffet lunch will be available to those who RSVP

[2] Presenters should arrive no later than 1:00 pm to their designated room to upload their presentations. Presentations should be 12-minutes and allow 3 minutes for questions and answers.

[3] We invite all attendees to RSVP join us for the Award ceremony at the BI auditorium.

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Applied/Clinical Science ORAL PRESENTATIONS
Phi Zeta Research Day
March 5, 2024, 1:15 – 4:30 pm
BI Auditorium

1:15 – 1:30	Lorelei Bojorquez	Multiple peripheral arteriovenous malformations in a dog
1:30 – 1:45	Jiashi Feng	Ocular and vulvar squamous cell carcinoma in a 17-year-old Holstein Friesian cow in the Caribbean
1:45 – 2:00	Isaac Fitz	Surveillance for Zoonotic Pathogens in Wastewater
2:00 – 2:15	Katie Long	Efficacy of Concomitant Antimicrobial Therapies for the Treatment of Bovine Respiratory Disease: A Systematic Review of the Literature
2:15 – 2:30	Kamilyah R. Miller	The Hardship of Parasite Diagnostics: Comparing Two Fecal Techniques for the Detection of Echinococcus multilocularis in wild canids
2:30 – 3:00	**Break for Poster Session**[1]	
3:00 – 3:15	Trey Neyland	Multicentric Lymphosarcoma in a 2.5-year-old Nigerian Dwarf Wether Goat
3:15 – 3:30	Jacob Schumacher	Evaluating the efficacy of Maternal Bovine Appeasing Substance (MBAS) (FerAppease®) administration on pain outcomes in calves after cautery dehorning and surgical castration
3:30 – 3:45	Anastasia Towe	Acute abdominal distension, hypersalivation, and pale mucous membranes in a Spotted-Hyena (Crocuta crocuta)
3:45 – 4:00	Tori Trumble	Pharmacokinetics of Cannabinoids Administered Orally with and without Food in Hispaniolan Amazon Parrots (Amazona ventralis)
4:00 – 4:15	Astrid Carcamo Tzic	Correlation of antinociceptive and hypothermic effects of opioids in dogs
4:15 – 4:30	Caylie D. Voudren	Gastrointestinal Microbiota Dysbiosis Contributes to Plasma Glucagon-like Peptide-2 Variability between Dogs with Chronic Enteropathies and Healthy Dogs

[1] Poster presentations will be held at the **Atrium of the BI Auditorium**

Applied/Clinical Science ORAL PRESENTATIONS
Phi Zeta Research Day
March 5, 2024, 1:15 – 4:30 pm
201 Trotter Hall

1:15 – 1:30	Jessica Carnal	Evaluating firocoxib, bupivacaine liposome suspension, and/or a CO2 surgical laser to improve piglet welfare
1:30 – 1:45	Rachel Champagne	Association of liver abscess with demographic factors, comorbidities, and gastrointestinal histologic morphology in feedlot mortalities
1:45 – 2:00	Thomas A. Christensen II	YAVSAP: versatile viral quasispecies analysis for veterinary samples
2:00 – 2:15	Stephen Edache	Assessing the impact of a postbiotic on Salmonella enterica prevalence, serotype diversity, and antimicrobial resistance in subiliac lymph nodes of cull dairy cattle
2:15 – 2:30	Luis F. B. B. Feitoza	Novel Point-of-Care Ultrasound Method Unveils Potential Imaging Parameters for differentiating Feedlot Respiratory Diseases
2:30 – 3:00	**Break for Poster Session**[1]	
3:00-3:15	Vanessa Horton	Swine Chronicles: Unlocking the Secrets of Japanese Encephalitis Virus
3:15 - 3:30	Alyssa Leslie	Evaluation of a needle-free injection system for administration of cloprostenol for luteolysis in lactating dairy cows
3:30 – 3:45	Madeline Mancke	Evaluation of cardiac troponin as a predictor of clinical outcome in cattle treated for bovine respiratory disease (BRD) in commercial feedyards
3:45 – 4:00	Taylor McAtee	Quantifying the Impact of Feedlot Cattle Performance on Greenhouse Gas Emissions
4:00 – 4:15	Megan McGraw	Effect of Seasonal Prescribed Burning on Muscid Fly Populations on Cattle Gazed in the Kansas Flint Hills
4:15 – 4:30	Trey Neyland	The Effect of Flunixin Meglumine on Viral Shedding in Calves

[1] Poster presentations will be held at the **Atrium of the BI Auditorium**

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Basic Science ORAL PRESENTATIONS
Phi Zeta Research Day
March 5, 2024, 1:15 – 4:30 pm
301 Trotter Hall

1:15 – 1:30	Konner Cool	Ferrets as a Model for SARS-CoV-2 Infection During Pregnancy
1:30 – 1:45	Douglas Farleigh	Preliminary analysis of canine cancer cases and environmental contaminants in Kansas (2021-2022)
1:45 – 2:00	Chandler Hansen	Assessing Veterinarian's Perspectives on the Applicability of Anatomy Education to Clinical Practice
2:00 – 2:15	Dongoh Lee	Enhancement of chloride versus bicarbonate conductance by CFTR modulators
2:15 – 2:30	Daniel Madden	Immune dysregulation at the cellular, cytokine, and transcriptomic level during African swine fever virus infection
2:30 – 3:00	**Break for Poster Session**[1]	
3:00 – 3:15	Chester D McDowell	Development of long-read targeted whole genome sequencing for African and classical swine fever viruses.
3:15 – 3:30	Theresa A. Quintana	Characterization of ABC transporter genes in Toxocara canis using RNA-Seq.
3:30 – 3:45	Cameron Sutherland	Evaluation of Environmental Bartonella spp. in Flea-Infested Homes
3:45 – 4:00	Alexandria Zabiegala	Comparative analysis of various host proteases in the entry of SARS-CoV-2, SARS-CoV, and MERS-CoV using the pseudovirus assay

[1] Poster presentations will be held at the **Atrium of the BI Auditorium**

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Applied/Clinical Science POSTERS
Phi Zeta Research Day
March 5, 2024, 2:30 – 3:30
BI Atrium

(Posting from 1:00 – 4:30 pm; Q&A for Judging 2:30 – 3:30 pm)

1	Danielle Lopez	Can Anxiety Fog Your Memory? An Alzheimer's Disease Animal Model
2	Deanna Gennett	Evaluating estimated carbon emissions and feedlot production data to address beef sustainability
3	Elayna Anderson	Can Florfenicol be a Substitute for Chloramphenicol in Resistant Canine UTIs?
4	Gabrielle Maroulis	Observational Assessment of Water Use in a Large Animal Teaching Hospital
5	Greta Karwath	Pharmacokinetic comparison and bioequivalence of emodepside formulations in dogs
6	Grace Schieferecke	Molecular Analysis of Benzimidazole Resistance in Hookworms in Kansas Dogs
7	Haitham Alneaemy	Lysine and lactate, alone or in combination, as substrates for Fusobacterium necrophorum in an in vitro ruminal fermentation system
8	Harith Salih	Antibacterial activities of grape seed, green tea and rosemary phenolic extracts against pathogens that cause liver abscesses in feedlot cattle
9	Mina Abbasi	Liver abscesses in feedlot cattle: Is Fusobacterium necrophorum subsp. necrophorum concentrations in ruminal and colonic epithelial tissues related to occurrence of liver abscesses?
10	Mikaela Weeder	Comparison of firocoxib, meloxicam and transdermal flunixin for pain mitigation in meat goats with experimentally induced lameness
11	Rebecca Bigelow	Evaluating the efficacy of Maternal Bovine Appeasing Substance (MBAS) (FerAppease®) administration on performance outcomes in bottle-fed beef-on-dairy calves during weaning

Basic Science POSTERS
Phi Zeta Research Day
March 5, 2024, 2:30 – 3:30 pm
BI Atrium

(Posting from 1:00 – 4:30 pm; Q&A for Judging 2:30 – 3:30 pm)

1	Cesar Aparicio	Microwave-responsive thermosensitive lipid nanoparticles for spatiotemporal delivery of chemotherapeutics
2	Erin Mayhue	Interaction of emodepside with canine MDR1: in silico and cellular assay
3	Hanna Rhoads	Got Lyme? Prevalence of Borrelia burgdorferi in Ixodes scapularis ticks collected from horses across the United States
4	Mahamudul Hasan	Online Survey on Canine Infectious Respiratory Disease Complex: Risk Factor, Tracking and Disease Pattern History from Dog Owners across the United States of America

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5	Mehrnaz Ardalan	Determination of seroprevalence for SARS-CoV-2 in farmed and wild white-tailed deer
6	Mehrnaz Ardalan	Determination of SARS-CoV-2 seroprevalence in cattle, sheep, and goats using different ELISA tests
7	Sarah DeVader	Water extract from Euglena gracilis attenuates lung cancer growth in mice by increasing PD-1+ CD8+ effector tumor infiltrating lymphocytes
8	Brian Wolfe	Biological sex differences in disease severity, lethal doses, and antibody responses after infection with H1N1 and H3N2 influenza A viruses in a mouse model
9	Saurav Pantha	Establishment of a mouse model of diet-induced obesity to study the effects of interaction of biological sex and obesity during influenza A virus pathogenesis

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ORAL PRESENTATIONS REQUIREMENTS:

- Arrive no later than 1:00 pm to your designated room to upload your presentation.
- No specific format required.
- 12-minute presentation time limit; 3 minutes allowed for questions and answers.
- Please give 24-48 hours advance notice if you are unable to attend/present due to unforeseen circumstances.

POSTER PRESENTATION REQUIREMENTS:

- Poster Size: The supplied poster boards can accommodate posters 48 inches wide by 36 inches deep.
- Poster PDFs need to be sent to Anna Harris (annak27@vet.k-state.edu) by **March 1, 2024**.
- Posters can be printed with the Veterinary Medical Library's Print Graphic Services. Contact Susie Larson at 785-532-4025 or larson@vet.k-state.edu. The cost of poster printing is the responsibility of the presenter or presenter's mentor.
- The BI Atrium will be open from 9:00 am on the day for set-up.
- Table tents and thumbtacks will be provided.
- The Poster Session question and answer time for judging will be from **2:30-3:30 pm.**
- Posters must be removed from the BI Atrium by **5:00 pm**
- Please give 24-48 hours advance notice if you are unable to present due to unforeseen circumstances.

PHI ZETA OFFICERS 2024:

On behalf of the Phi Zeta Executive Committee (2024):

Dr. Raghavendra Amachawadi – President (agraghav@vet.k-state.edu)
Bailey Fritz – Vice President (brfritz@vet.k-state.edu)
Dr. Nicolette Cassel – Secretary/Treasurer (ncassel@vet.k-state.edu)
Anna Harris – Alumni Affairs & Events (annak27@vet.k-state.edu)
Diane Beets – Executive Assistant (dmhess@vet.k-state.edu)

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Presenter: Lorelei Bojorquez

Multiple peripheral arteriovenous malformations in a dog

Corresponding Author: Lorelei Bojorquez, MVM DVM. Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University

Co-Authors: Darby Toth, DVM, Nicolette Cassel, BSc, BVSc, MMedVet, Dip ECVDI, Clay Hallman, DVM, DACVR

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Keywords: Arteriovenous malformations; Vascular anomalies; Peripheral; Congenital; Dog

Abstract

Arteriovenous malformations (AVMs) and fistulas (AVFs) are a subset of the diversity of vascular anomalies that have been described in humans and animals. The peripheral presentation of AVMs and AVFs is uncommon, and, in the veterinary literature, case reports are rare.

A 4.5-month-old dog presented for a 2-month history of progressive left hind limb lameness and pain. Physical exam revealed minimal weight bearing, tarsal valgus and swelling of the stifle. A strong pulse in the left femoral and saphenous veins was noted, with a palpable thrill extending distally to the metatarsus.

Radiography, ultrasound, and computed tomography led to the detection of three plexuses of abnormal blood vessels that were consistent with arteriovenous malformations or fistulas, located within the left middle gluteal muscle, the cranial tibial muscle and the left metatarsus, respectively. There was associated polyostotic involvement, evidenced by multifocal expansile lysis and cortical thinning with numerous enlarged vascular channels along the distal femur, tibia and metatarsus.

A surgical approach to the caudal abdomen to ligate the feeding vessels to the plexus in the middle gluteal was performed, and the left internal iliac artery and vein were ligated. Following internal iliac ligation, a left hind limb amputation was performed without complications and the patient recovered uneventfully. No post-operative complications associated with internal iliac artery and vein ligation were observed.

Histopathological findings of the amputated limb were consistent with arteriovenous malformations or a type of angiomatosis.

Arteriovenous malformations and fistulas are a subset of high flow vascular malformations and the terms are often used interchangeably in veterinary medicine. AVMs are comprised of a nidus of tangled vessels with a single artery or arteries that communicate directly with a single vein or multiple veins. In contrast, AVFs lack a nidus and consist of a direct arteriovenous communication. However, differentiating them may be difficult because multiple arteriovenous fistulas may be present at one lesion.

Clinical signs result from local ischemia and elevated venous pressures leading to edema, dilatated and tortuous veins, swelling, pain, and even ulcerative lesions. Long-term consequences of these vascular anomalies may include life-threatening hemorrhage and high-output cardiac failure.

Imaging is essential in the diagnostic process, and magnetic resonance angiography is the gold standard screening method. However, computed tomography angiography can also provide a comprehensive vascular map and has the advantage of shorter acquisition times.

In human medicine, treatment options range from monitoring over time to complex vascular microsurgeries. Although interventional procedures are technically challenging and may be cost-prohibitive in the veterinary practice, there are some reports of successful outcomes. Wide surgical excision and limb amputation are also feasible alternatives in many cases.

Peripheral congenital AMVs and AVFs are rare but should be considered as a differential diagnosis in young dogs with clinical signs such as lameness, soft tissue swelling, pain and a palpable thrill in peripheral veins. A thorough understanding of the nature, anatomy and consequences of these vascular anomalies is required to appropriately diagnose and successfully manage these cases.

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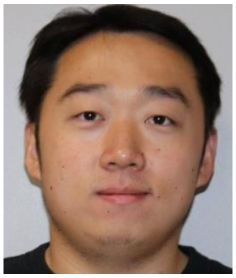

Presenter: Jiashi Feng

Ocular and vulvar squamous cell carcinoma in a 17-year-old Holstein Friesian cow in the Caribbean

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Keywords: bovine; ocular squamous cell carcinoma; immunohistochemistry

Abstract

Introduction: Bovine ocular squamous cell carcinoma (SCC), or ‘cancer eye’, is the most common malignant tumor affecting cattle in North America and is a leading cause of whole-carcass condemnation at slaughter in the US. The risk factors for developing bovine ocular SCC include, but are not limited to, ultraviolet light exposure, facial hypopigmentation, viral infection (bovine herpesvirus 1, and bovine papillomaviruses), age, and breeds such as Hereford.

Case Description: A 17-year-old, approximately 700-kg Holstein Friesian cow from the teaching herd at a veterinary school in the tropical region was presented with a chronically swollen, unable-to-open left eye and slow-growing vulvar and anal masses causing a perineal deformation. Medical therapy was initially prescribed to treat the eye condition for a suspicion of panophthalmitis. After treatment failure and further evaluation, the left eye was exenterated and submitted for biopsy. A vulvar mass was surgically excised and submitted for biopsy at a later date. A diagnosis of ocular and vulvar SCC was made, confirmed with immunohistochemistry (cytokeratin, vimentin, Melan A, and desmin). Approximately 12 months following the exenteration, the cow deteriorated with purulent nasal discharge and signs of suspected tumor local invasion and metastasis (multiple lymph node

enlargements). Following euthanasia and autopsy, macroscopic and microscopic evaluation revealed the left maxillary sinus contained SCC, with secondary necro-ulcerative sinusitis, likely an extension from the ocular SCC. The aerobic culture of samples from the purulent material revealed the organism Trueperella pyogenes. On histologic evaluation, collected lymph nodes appeared reactive, without evidence of tumor metastasis. Immunostaining for bovine papillomavirus was negative on submitted sections from the ocular and vulvar masses. Other notable findings include pheochromocytoma, myometrial leiomyoma, chronic interstitial nephritis, and Sarcocysts sp. cysts in the myocardium.

Discussion: This present case is a geriatric Holstein Friesian cow with multiple neoplastic diseases, including ocular and vulvar SCC, myometrial leiomyoma, and pheochromocytoma. Questions had arisen regarding whether the two SCCs (ocular and vulvar) occurred independently, due to metastasis, or shared a common viral etiology. Due to the lack of evidence for metastasis (lymphovascular invasion) and viral infection, the ocular and vulvar SCCs were considered independent primary tumors. However, metastasis was still possible. Viruses could still have played a role in the initiation phase of the neoplastic transformation at an earlier age and simply became latent. The animal had dark periorbital pigmentation around the affected eye, and the prolonged exposure to sunlight from living in a tropical region and old age could explain the development of ocular SCC despite protective periorbital pigmentation against UV light exposure. This case report highlights several neoplastic diseases in domestic cattle and associated risk factors, and it also emphasizes the importance of histopathology and immunohistochemistry in diagnosing suspected neoplasms.

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Presenter: Isaac Fitz

Surveillance for Zoonotic Pathogens in Wastewater

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Keywords: Epidemiology; Zoonotic; Surveillance

Abstract

Zoonotic pathogens pose significant risks to human and animal health. Monitoring the presence of zoonotic pathogens in the environment is a valuable epidemiological tool and important for pandemic preparedness and prevention. Environmental samples such as wastewater and runoff samples are easily accessible for pathogen surveillance in both human and animal populations and can be easily scaled up to meet testing needs.

In this study, we aimed to determine the presence of various zoonotic pathogens in wastewater near livestock facilities and natural waterways. Sequential wastewater samples from eight different locations were collected from May to August 2023: rivers or waterways (5 sites; 41 samples), and sanitary sewer sites from three agricultural locations (3 sites; 66 samples). The wastewater samples were tested for viral (SARS-CoV-2, Influenza A and Mpox) and bacterial (E. Coli 0157, Salmonella sp., Leptospira sp.) zoonotic pathogens.

Wastewater samples were processed as polyethylene glycol precipitated pellets that were transferred to transport media and stored at -80°C until testing. Samples were thawed, vortexed and gravity sedimentation occurred overnight at 4°C. Total nucleic acid (NA) extraction was performed using the GeneReach DNA/RNA extraction kit with minor modifications for water testing. Samples were prediluted 1:1 in PBS and 200ul was extracted along with an exogenous control DNA fragment (XENO DNA) for monitoring for inhibitors. Extraction was performed on an automated magnetic bead processor using extraction positive (Influenza A RNA and Mpox DNA) and negative (water) controls on each extraction plate. Previously published real time PCR (qPCR) or reverse transcription qPCR (RT-qPCR) assays were used to detect pathogen-specific viral and bacterial NA; reactions were set up in duplicate for each sample. Positive samples were defined as those with both replicates with a Ct less than 43; suspect samples were designated as those with single positive wells. qPCR/RT-qPCR assays were validated using established pathogen-specific controls prior to sample testing.

For wastewater samples near livestock, 6% tested positive/suspect for SARS-CoV-2, 1.5% were positive/suspect for Influenza A, 71% were positive/suspect for E. coli O157 and 46% were positive/suspect for the Salmonella sp., while 20% were positive/suspect for the S. typhimurium. Lastly, 46% of livestock wastewater samples were positive/suspect for the Leptospira 16S DNA. In contrast, aside from the detection of Leptospira 16S DNA in 51% of the waterway samples, only two waterway samples tested suspect for Influenza A; the remainder were negative for all the other pathogens tested.

This preliminary study demonstrates that wastewater surveillance collected from sanitary sewers near livestock facilities in Oklahoma is a viable method for monitoring zoonotic pathogens. The results obtained are relevant for epidemiological studies, pathogen risk assessment, as well as pandemic preparedness and prevention regarding zoonotic pathogens potentially originating from intense agriculture or wildlife.

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Presenter: Katie Long

Efficacy of Concomitant Antimicrobial Therapies for the Treatment of Bovine Respiratory Disease: A Systematic Review of the Literature

Corresponding Author: Brian Lubbers, DVM, PhD, Beef Cattle Institute, Kansas State University College of Veterinary Medicine, Manhattan, Kansas, U.S.A.

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Keywords: Concomitant antimicrobials, combination therapy, bovine respiratory disease, BRD

Abstract

Many feedlots administer antibiotics for treatment of bovine respiratory disease (BRD). Refractory animals will often be retreated with a different class of antibiotics for BRD. As combination therapy becomes more prevalent within the beef industry, knowing the impacts it may have on animal welfare, antimicrobial resistance and antimicrobial stewardship is crucial. We hypothesized that combination antimicrobial therapy is not significantly different than solo antimicrobial therapy.

A literature search, using the PubMed database, was conducted in July of 2023 to find evidence in support of combination antimicrobial therapy. The cattle search string included: feedlot cattle, or bovine, or cattle; and bovine respiratory disease, or BRD, or bovine respiratory disease complex; and synergism, or synergy, or concomitant therapy, or combination antimicrobial therapy, or combination therapy, or combination antibiotic therapy. Published reports were included in the literature summary if they used a combination therapy treatment group with comparison of treatment outcome to a monotherapy treatment that included at least one of the combination drugs. Published reports were not further considered if the combination antimicrobials were not administered concurrently, or if the product was a fixed drug combination (ex: Sulbactam).

Due to the paucity of literature from the cattle search, an expanded parallel search was performed on human medical literature, also utilizing the PubMed database. The human medical search string included similar terms to the cattle search string. The human search was limited to clinical trials specific

to respiratory disease published between 1980-2023, written in English, and did not include cancer or COVID.

The cattle search string returned 295 articles. The human search string returned 376 articles. After abstract review, article evaluation, and application of inclusion criteria, 6 relevant cattle articles and 2 relevant human articles were left for final review.

The paucity of literature does not provide enough evidence to support or refute the efficacy of concomitant therapy. However, of the few available studies, no significant advantage or disadvantage regarding in vitro measures or treatment outcomes between combination therapy and solo therapy.

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Presenter: Kamilyah R. Miller

The Hardship of Parasite Diagnostics: Comparing Two Fecal Techniques for the Detection of Echinococcus multilocularis in wild canids

Corresponding Author: Kamilyah R. Miller, DVM, Kansas State University College of Veterinary, Manhattan, KS, USA

Co-Authors: Briana Raya, Kansas State University College of Veterinary Medicine, Manhattan, KS, USA; Brian H. Herrin, DVM, PhD, DACVM (Parasitology), Kansas State University College of Veterinary Medicine, Manhattan, KS, USA

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Keywords: Wildlife, Parasitology, Diagnostics

Abstract

Background: Echinococcus spp. are zoonotic tapeworms of wild canids that also infects humans and animals. The range of Echinococcus spp. in the United States is expanding through the coyote population, warranting further investigation of wild canids and domestic dogs in non-endemic areas. However, diagnosing Echinococcus spp. infections, in general, is difficult. The gold standard for diagnosing Echinococcus spp. infections by finding adults in the small intestine by purging or through necropsy. Still, this method is labor and time-intensive, and not practical in a clinical setting. Thus, a need for a test with the same sensitivity and specificity as adult Echinococcus spp. recovery is needed.

Methods: Coyote carcasses were collected from Kansas (n=53) and Missouri (n=52). Intestinal tracts were removed, frozen at -80°C for at least 7 days, thawed, and then processed by sifting, filtration, and counting technique (SFCT) to identify adult Echinococcus spp. Feces were collected during SFCT from the distal half of the intestinal tract, preserved in 70% Ethanol, and stored at 4°C until testing. For all fecal samples, PCR was done targeting the nad1 gene for E. multilocularis then, a centrifugal sugar flotation was used to identify taenia-type eggs. The evidence of any other parasite eggs was recorded.

Results: Echinococcus spp. adults were recovered from 41% (KS = 25/53; MO = 19/52) of coyotes collected. All positive samples were morphologically, and molecularly, confirmed as E. multilocularis with sequences closely matching European isolates. We detected Echinococcus spp. DNA in 29% (KS = 11/50; MO = 8/51) of fecal samples. All fecal PCR samples were confirmed as E. multilocularis, closely matching European isolates. When comparing fecal PCR to adult Echinococcus spp. recovery, the gold

standard, fecal PCR identified only 34% (KS = 9/25; MO = 6/19) of positive samples. We found taenia-type eggs on fecal flotations in 27% (KS = 13/50; MO = 15/51) of samples with molecular confirmation pending. Molecular confirmation is needed because both Taenia spp. and Echinococcus spp. tapeworms produce taenia-type eggs. In addition to taenia-type eggs Alaria sp., Physaloptera sp., and Trichuris sp., were detected. In our study, fecal PCR was shown to be more effective at diagnosing Echinococcus spp. infections than standard fecal flotations.

Conclusion: Echinococcus spp. adult recovery remains the gold standard for definitively diagnosing infections. As peri-urban coyote populations continue to pose a zoonotic threat to humans and domestic animals, the demand to find a time-efficient and reliable screening method is needed for domestic dogs and wild canids.

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Presenter: Trey Neyland

Multicentric Lymphosarcoma in a 2.5-year-old Nigerian Dwarf Wether Goat

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Keywords: Lymphosarcoma; Caprine; Goat; Magnetic resonance imaging

Abstract

Introduction: A 2.5-year-old Nigerian dwarf wether goat presented to the Kansas State Veterinary Health Center for acute duration of inability to stand.

Case Presentation: Initial physical exam revealed a wide base stance when assisted to stand, decreased conscious proprioception of the hind limbs and normal to decreased patellar reflexes bilaterally. The spinal radiographs performed did not show boney changes affecting the vertebral column. Cerebrospinal fluid revealed a hyperproteinemia and mononuclear pleocytosis. Other diagnostics showed the patient had a left shift neutrophilia and multiple electrolyte derangements. The patient was started on anti-inflammatories, antibiotics, and physical therapy. Six days post presentation, the patient regressed neurologically and was unable to stand. Magnetic resonance imaging was performed revealing an L3-L4 extramedullary mass like lesion associated with severe compressive myelopathy.

Conclusion: The patient was euthanized and submitted for necropsy. Necropsy revealed a multicentric T-cell B-cell rich lymphosarcoma affecting the spinal cord, heart, abomasum, and prostate.

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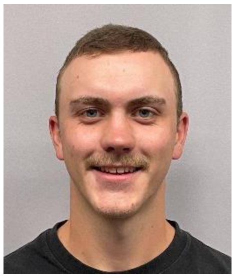

Presenter: Jacob Schumacher

Evaluating the efficacy of Maternal Bovine Appeasing Substance (MBAS) (FerAppease®) administration on pain outcomes in calves after cautery dehorning and surgical castration

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Keywords: FerAppease;castration;cattle;calves;welfare

Abstract

Castration and dehorning are standard management practices in cattle industry, causing pain and distress. Currently, in the U.S., no analgesic or anesthetic drugs are approved by the FDA to relieve pain in calves undergoing castration and dehorning. Maternal Bovine Appeasing Substance (MBAS) is naturally secreted by the sebaceous gland of the mammary gland and induces a calming effect in nursing calves. This project aims to determine if the addition of a single topical dose of MBAS (FerAppease®, FERA diagnostics and Biologicals, TX) or in combination with a systemic analgesic will provide extended pain and stress relief to calves after hot iron dehorning and castration. Forty-nine Holstein bull calves, 1-2 weeks of age, weighing approximately 50 kg, were enrolled in this project. Calves were randomly assigned to one of the following treatment groups: lidocaine local anesthesia (LIDO; n=9), lidocaine and systemic analgesic meloxicam (LIDO-MEL; n=10), lidocaine and MBAS (LIDO-MBAS; n=10), (4) lidocaine and meloxicam and MBAS (COMBO; n=9), sham procedures (SHAM; n=6); and no analgesia (CONTROL; n=5). Calves' body weight, behavior, mechanical nociception threshold, gait, thermography, blood cortisol, and substance P concentrations were assessed. A linear mixed model with fixed effects of time, treatment, and treatment × time was used to analyze performance variables. Calf and cohort were used as a random effect. No significant weight and average daily gain differences were observed between treatments (P>0.05). Further analysis of other outcomes is ongoing. The welfare benefits of calves undergoing dehorning and castration will be verified upon analysis of the remaining outcomes.

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Presenter: Anastasia Towe

Acute abdominal distension, hypersalivation, and pale mucous membranes in a Spotted-Hyena (Crocuta crocuta)

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Co-Authors: Tori Trumble, DVM, Brandon Plattner, DVM, PhD, Rochelle Day, DVM, Trenton Shrader, DVM (all Kansas State University)

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Keywords: hyena, neoplasia, carcinomatosis, immunohistochemistry

Abstract

A 15-year-old, female spayed, spotted hyena (Crocuta crocuta) weighing 61.7kg presented to the Kansas State University College of Veterinary Medicine's Exotic and Zoological Medicine Service for distended abdomen, dyspnea, and hyporexia. Bloating and hyporexia were first appreciated 48 hours prior to presentation. The following day, she progressed to complete inappetence. The morning of presentation, she displayed a hunched posture, muscle fasciculations, ptyalism, dyspnea, and pale mucous membranes. This acute progression prompted emergent immobilization and evaluation. The hyena was chemically immobilized and put under general anesthesia for transport to the hospital. Ultrasound revealed mild pleural effusion in the left cranial thorax, abundant abdominal fluid, and irregular, nodular, intermittently hypoechoic areas within the left mesentery/omentum. Abdominal exploratory surgery was pursued, and due to the finding of diffuse, presumed neoplastic nodules throughout the abdominal cavity, euthanasia was elected. A postmortem examination was performed.Through gross necropsy and histopathology, the hyena was diagnosed with adenocarcinoma, presumed pyloric in origin, with widespread abdominal and thoracic parietal intercostal pleural carcinomatosis, and extensive scirrhous response. Reports of epithelial neoplasia in hyenas are sparse, with a single previous report of squamous cell carcinoma of the anal sac, and no prior reports of adenocarcinoma. Similarly, there are few reports of gastrointestinal neoplasia in this species, with only one case of intestinal lymphosarcoma described in the literature. Immunohistochemistry was vital in this case for distinguishing between neoplastic cells and mesothelial hyperplasia.

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Presenter: Tori Trumble

Pharmacokinetics of Cannabinoids Administered Orally with and without Food in Hispaniolan Amazon Parrots (Amazona ventralis)

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Keywords: pharmacokinetics; cannabinoids; CBD; avian; Hispaniolan Amazon parrot

Abstract

Introduction: The receptors of the endocannabinoid system are highly conserved across species and are upregulated in states of inflammation, immunosuppression, and neoplasia. Phytocannabinoid compounds, such as cannabidiol), have been shown to have potent anti-inflammatory, analgesic, antimicrobial, anti-convulsant, anti-emetic, and anti-anxiety effects. These properties make cannabidiol an attractive potential therapeutic option in veterinary medicine. In a previous study, cannabidiol was administered to 14 healthy, Hispaniolan Amazon parrots (Amazona ventralis) at doses of either 60 mg/kg or 120 mg/kg per os in a fasted state. The results of which were overall variable with a short half-life of 1.28 hrs even at 120 mg/kg. Phytocannabinoids are highly lipophilic and undergo first-pass metabolism. Literature from human, canine, and primate studies has demonstrated that oral administration with a high-fat meal significantly increases the bioavailability and serum concentrations of cannabidiol compared to ingestion in a fed state. The purpose of this study was to compare the pharmacokinetics of cannabidiol with and without food in the Hispaniolan Amazon parrot.

Methods: In this study, 12 healthy, Hispaniolian Amazon parrots of mixed sex weighing between 0.260-0.323 kg were enrolled. Six birds were administered 120 mg/kg cannabidiol per os followed by 15 ml/kg of a slurry formulated diet, and 6 birds were administered 12 0mg/kg cannabidiol per os without a food slurry bolus. Blood samples were obtained at time 0, and at 1, 2, 4, 6, 8, and 12 hours post-treatment in

a balanced incomplete block design. Sample analysis will be determined using a high-performance liquid chromatography (HPLC)/mass spectrometry system. Values of pharmacokinetic variables will be determined by use of noncompartmental analysis performed with commercially available software (PKSolver, Microsoft Corp). The following variables will be determined: area under the plasma versus time curve (AUC); area under the first moment curve (AUMC); terminal rate constant (λ); terminal half-life (t½) (hr); observed maximum concentration (Cmax); and time to reach Cmax (Tmax). Values for AUC and AUMC will also be determined.

Results: The results are currently under analysis. The area under the curve (h_ng/ml), Cmax (ng/ml), and times to achieve Cmax (hours) for the slurry bolus and no bolus groups, respectively, and the serum half lives are currently being determined.

Conclusion: The results of this study will determine if the administration of cannabidiol with a food slurry bolus increases the bioavailability of cannabidiol in parrots as it does in various species other than the rabbit.

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Presenter: Astrid Carcamo Tzic

Correlation of antinociceptive and hypothermic effects of opioids in dogs

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Keywords: analgesia; animal; preclinical; refinement; welfare.

Abstract

The purpose of this study was to evaluate the analgesic effects of butorphanol and methadone using a thermal pad model, and to assess the correlation of analgesia and hypothermia caused by opioids in dogs. Nine male beagles were enrolled and randomly allocated to receive intravenous methadone (0.5mg/kg), butorphanol (0.4mg/kg), or saline/placebo in an IACUC-approved three-way cross-over study. Rectal temperature and thermal withdrawals were measured prior to drug administration and through 6 hours post administration. Thermal withdrawals were obtained randomly from each hind foot, placed on thermal plates heated to a maximum of 134F (equivalent to 20 seconds) for each time point (3 per foot, 6 per dog). A positive withdrawal occurred if the dog actively removed the foot. One dog was excluded from analysis due to lack of withdrawals at maximum temperatures/times. No injuries were noted to the dogs' feet. Thermal withdrawals were significantly decreased (P<0.5), compared to saline, from 0.5-5h in methadone and 0.5-4h for butorphanol. Rectal temperatures were significantly decreased, compared to saline, from 0.5-5 hours for both methadone and butorphanol. The magnitude of the hypothermia was greater for methadone compared to butorphanol. There was significant (P=0.0005), and moderate correlation (r2=0.43) between the analgesic and hypothermic effects of the opioids. The dogs administered butorphanol and methadone exhibited sedation, but sedation was not specifically quantified. Based on these data, the hypothermic and analgesic effects of butorphanol and methadone were correlated. Monitoring opioid hypothermic effects is an easy, inexpensive, and humane manner for preclinical predictor of opioid analgesic effects in dogs.

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Presenter: Caylie D. Voudren

Gastrointestinal Microbiota Dysbiosis Contributes to Plasma Glucagon-like Peptide-2 Variability between Dogs with Chronic Enteropathies and Healthy Dogs

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Keywords: Enteroendocrine, Dysbiosis, Gastrointestinal, Inflammatory Bowel Disease, Microbiome

Abstract

Introduction: Chronic enteropathies (CE) are associated with disruption of the normal gastrointestinal mucosal barrier and microbiome. Plasma glucagon-like peptide-2 (GLP-2), a hormone responsible for the mucosal barrier structure and repair, is decreased in dogs with CE. As microbial-mucosal interactions and metabolic by-products, stimulate GLP-2 secretion, association between microbiota and GLP-2 concentrations could further support the microbiome as a target for canine CE management.

Methods: In this prospective study, plasma GLP-2 concentrations (ELISA), as well as targeted (dysbiosis index) and untargeted 16S V4 rRNA fecal microbiome analyses were performed on healthy controls and CE dogs prior to and 1 month following CE-directed treatment. Diversity indices and bacterial population abundances were compared between groups and time-points. Principal component analysis combined with least squares regression was used to identify taxa contributing to GLP-2 variance among groups.

Results: While the dysbiosis index did not differ between healthy and CE dogs, untargeted sequencing identified 47 OTUs that differed between the groups, all but 2 of which resolved with treatment. Six families and 19 genera contributed to differences in GLP-2 concentrations between groups.

Conclusions: Altered GI microbial populations may contribute to decreased circulating GLP-2 in canine CE. The association between increased plasma GLP-2 and dysbiosis recovery supports the importance of microbiota in GI health. Further research into mechanisms of microbiota impact on the enteroendocrine system is needed.

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Presenter: Jessica Carnal

Evaluating firocoxib, bupivacaine liposome suspension, and/or a CO2 surgical laser to improve piglet welfare

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Keywords: welfare; piglets; firocoxib; castration; tail docking

Abstract

Each year, piglets in the U.S. are surgically castrated and tail docked without pain management. Exploring innovative surgical techniques and drug delivery methods are essential to improve welfare. This study aimed to evaluate the effectiveness of a CO2 surgical laser and a bupivacaine liposome suspension (BUP), used alone or in conjunction with firocoxib (FIRO), on reducing piglet pain and improving wound healing after surgical castration and tail docking. We hypothesized that the combined use of FIRO, BUP, and the surgical laser would significantly reduce pain and improve wound healing when compared to current industry standards. Three sows, each with a litter of ten piglets (n=5 male and 5 female), were randomly assigned to one of two treatment groups: 3.0mg/kg FIRO oral (n=1 sow), or oral placebo (n=2 sows); therefore, piglets received either FIRO (n=10) or placebo (n=20) via the sow through transmammary delivery. Additionally, the piglets of the placebo sows were either provided 3.0mg/kg FIRO oral (n=10 piglets), or an oral placebo (n=10 piglets). All piglets received BUP intratesticularly and/or at the base of the tail approximately 30min before processing. Piglets were processed using one of three options: surgical laser (n=12), scalpel and side pliers (conventional; n=12), or sham (handled only; n=6). Wound healing and inflammation were assessed at baseline and up to 168h post-processing. Tail and scrotal wound scores were lower in piglets that received FIRO transmammary compared to placebo ($p \leq 0.05$). Scrotal wound scores were also lower in piglets castrated using the laser ($p=0.004$), while tail wound scores were lower in piglets tail docked conventionally ($p=0.003$). Inflammation of the tail tissue was higher in piglets docked using the laser ($p=0.02$) when compared to piglets docked conventionally. Tail tissue inflammation was also seen to be higher in sham handled piglets that received either oral FIRO ($p=0.006$) or transmammary FIRO ($p=0.02$) when

compared to sham handled piglets in the control group. Piglets in the control group had higher cortisol blood plasma concentrations than piglets that received oral FIRO (p<0.0001), and piglets that received transmammary FIRO ($p=0.007$). Furthermore, piglets given transmammary FIRO had higher cortisol blood plasma concentrations ($p=0.01$) than piglets given oral FIRO. FIRO blood plasma concentrations did not change significantly from 1h to 24h for either treatment group. Preliminary results suggest that the surgical laser may improve wound healing when used for castration, but not for tail docking. The increased tail tissue temperatures of sham handled piglets given FIRO, when compared to sham handled piglets in the control group, may suggest that FIRO blocked the sympathetic nervous system (SNS) activation, since SNS activation from stress or pain leads to a decrease in skin temperature. Additionally, the lowered cortisol blood plasma concentrations of both FIRO treatment groups may indicate decreased stress in piglets during surgical castration and tail docking. FIRO was also demonstrated to be successfully delivered through transmammary delivery and remain stable in piglets for at least 24h. Further outcome analysis will be done to confirm these findings.

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Presenter: Rachel Champagne

Association of liver abscess with demographic factors, comorbidities, and gastrointestinal histologic morphology in feedlot mortalities

Corresponding Author:

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Keywords: Necropsy; Days on Feed; Sex; Gastrointestinal; Histopathology;

Abstract

Introduction: Liver abscesses are a major cause of hepatic condemnation in feedlot cattle at harvest (20% of beef carcasses affected). Carcass contamination, impaired feedyard performance, and antimicrobial stewardship are some of the major concerns surrounding liver abscess. Due to antemortem limitations in determining liver abscess status, risk factors of developing liver abscesses during the feeding phase are poorly characterized. The objective of this study was to identify associations of liver abscess at necropsy with comorbidity/demographic factors and gastrointestinal histologic morphology in feedlot mortalities.

Methods: Systematic necropsies (n=900) were performed in the central U.S. (six commercial feedyards; all of June/July during 2022 and 2023). Gross pathology was determined by necropsy technicians and confirmed by a veterinarian. Formalin-fixed sections of rumen, small intestine, and spiral colon were collected from liver abscess cases (n=40) and time-matched necropsies (no liver abscess) from the same feedyard (n=40) as controls. Demographic data collected including sex, days on feed at death (DOF), arrival weight, number of treatments, and estimated weight at death were collected from feedyard records. Morphologic features were measured in gastrointestinal tissues using hematoxylin and eosin-stained digitally scanned slides (Aperio eSlide Manager). Digitized alcian blue-stained slides were used to evaluate goblet cell area in small intestine and spiral colon tissues (ImageJ/Figi). General and linear mixed-effects models were used to evaluate liver abscess association with demographic/comorbidity factors and gastrointestinal morphologic differences between liver abscess and control cases. Multivariate models were developed using backward stepwise techniques where all variables with $P \leq 0.30$ from univariate models were included in the initial multivariate model and only variables with P < 0.10 were included in the final model.

Results: Steers had greater probability of liver abscess than heifers, 0.16 verses 0.06, respectively (P<0.01). Dairy/dairy-influenced animals had a higher probability (0.3) of liver abscess than traditional beef breeds (0.03) (P<0.05). Animals that were 101+ DOF had a higher probability of liver abscess than those that were <50 DOF (P<0.01). An animal having abdominal adhesions was positively associated with also having liver abscess (P<0.001). Liver abscess cattle had shallower crypts and thinner mucosa in the small intestine (P<0.05). There was a tendency (P<0.10) for liver abscess cattle to have a lower percent area of goblet cells in the small intestine, but there were no differences in spiral colon morphology.

Conclusions: Sex, breed, DOF, and abdominal adhesions were the only factors associated with liver abscess of all demographic/comorbidity factors included. In addition, multiple differences were observed in small intestine histologic morphology of liver abscess cattle compared to controls. Although results are biased to only feedlot mortalities, these findings indicate some association between liver abscess and post-rumen gut health. Further investigation is required to better understand factors influencing liver abscess formation in feedlot cattle.

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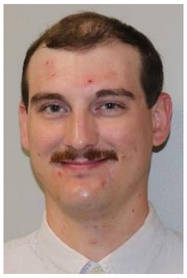

Presenter: Thomas A. Christensen II

YAVSAP: versatile viral quasispecies analysis for veterinary samples

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Keywords: virus; quasispecies; next-generation sequencing; pipeline

Abstract

Viral populations within an infected host are composed of viral particles with a spectrum of genetic mutations rather than a unified genome. This phenomenon is referred to as viral "quasispecies," and has been useful for the understanding of viral transmission and early detection of new viral variants. Next generation sequencing (NGS) has enabled the study of these quasispecies for many viral species, notably Influenza A and B, Human Immunodeficiency Virus (HIV), Foot and Mouth Disease Virus (FMDV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2), and established protocols and computer analysis tools have been developed for these species. Some of the most important viruses, such as emerging and exotic disease agents, however, do not have replicatable protocols or software tools capable of producing valid output from their sequence data. Here, we present Yet Another Viral Subspecies Analysis Pipeline (YAVSAP). YAVSAP is a fully automated bioinformatic pipeline built from the ground up to identify and analyze viral quasispecies of any arbitrary virus in human and veterinary samples. YAVSAP provides reference-based genome mapping of both long- and short-read sequencing reads to any reference genome that the user chooses, identifies subconsensus variants and haplotypes, and assesses the phylogenies of all viral sequences found within a sample. YAVSAP is written in Nextflow and conforms to the nf-core initiative's standards, which allows it to run on low-end computers, high performance computing (HPC) clusters, or anything in between with zero configuration. YAVSAP has been tested on viruses of interest to veterinary medicine and public health, including Japanese Encephalitis Virus (JEV), Influenza D Virus (IDV), Bovine Coronavirus (BCoV), SARS CoV2, and Rift Valley Fever Virus (RVFV), and can correctly identify

consensus genomes and quasispecies within samples containing each of these viruses. This tool provides a means for biologists with little bioinformatic experience to analyze deep sequence data while correcting for many of the pitfalls associated with previous and current analysis platforms. YAVSAP is open source software and is publicly available at https://github.com/ksumngs/yavsap.

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Presenter: Stephen Edache

Assessing the impact of a postbiotic on Salmonella enterica prevalence, serotype diversity, and antimicrobial resistance in subiliac lymph nodes of cull dairy cattle

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Keywords: Salmonella prevalence, subiliac lymph node, cull dairy cattle, postbiotic, food safety

Abstract

Background: Substantial evidence exists that cattle lymph nodes (LN) are a potential source of Salmonella in ground beef. However, since post-harvest interventions cannot effectively control this route of contamination and the complete removal of peripheral LN from beef carcasses is not practically feasible, there is a need to investigate pre-harvest mitigation strategies at the farm level. Given the existing, yet inconsistent, evidence indicating that dietary additives are one of the commercially available interventions that have proven effective in reducing Salmonella in cattle, the overarching objective of this study was to assess the impact of a postbiotic [XPC™ or Nutritek™ (Diamond V, Cedar Rapids, IA) – derived from Saccharomyces cerevisiae] on Salmonella prevalence, serotype diversity, and antimicrobial resistance in subiliac LN of cull dairy cattle harvested in two large commercial processing plants in the United States (U.S).

Methods: In collaboration with two commercial processing plants in the Southwestern (SW) and Northeastern (NE) regions of the U.S., cull dairy cattle lots processed on the same week from dairy farms that administered the postbiotic or did not feed the postbiotic (no-postbiotic) were identified and sampled. Up to 20 LN were collected from each supplier farm at least once every season. Samples were analyzed for Salmonella by culture and quantitative PCR methods. Confirmed isolates were subjected to serotype identification via classical serotyping and molecular typing, and to antimicrobial susceptibility testing using the broth micro-dilution method.

Results: A total of 2,816 LN were collected between May 28, 2021, and July 31, 2022, resulting in an overall Salmonella LN prevalence of 13.9% (391/2,816). Although a numerical reduction in prevalence was observed in the postbiotic farms (100/847, 11.8%) compared to the no-postbiotic farms (291/1,969, 14.8%), this reduction was not statistically significant (P = 0.485). Salmonella LN prevalence, however, significantly varied by region/processing plant (P < 0.001), where the SW region showed a higher prevalence (281/1,384, 20.3%) than its NE counterpart (110/1,432, 7.7%). Whereas dominant Salmonella serotypes included Montevideo, Mbandaka, Muenster, Cerro, Meleagridis, and Anatum, the probability of isolating a dominant serotype did not significantly vary by feed additive status (FAS) or region, however, it varied by season (P = 0.002). In terms of antimicrobial resistance, between 0 and 34 isolates (out of 391) exhibited resistance to each antimicrobial, with the highest number of isolates exhibiting resistance to streptomycin and ciprofloxacin, however, this did not significantly vary by FAS, season, or region.

Conclusion: Our findings show that although the cattle lots that received the postbiotic had a lower Salmonella LN prevalence than the no-postbiotic lots, the reduction was not statistically significant. Specific reasons for the lack of effectiveness of the postbiotic in significantly reducing the burden of Salmonella remains unclear; however, variables like the inconsistency in administration durations of the postbiotic due to culling of dairy cows at different stages of the lactation cycle or during dry periods may have limited its effectiveness. Further research is warranted to identify effective pre-harvest strategies to reduce the burden of Salmonella in cattle and mitigating the risk for subsequent Salmonella outbreaks.

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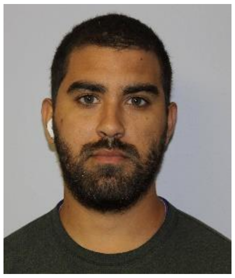

Presenter: Luis F. B. B. Feitoza

Novel Point-of-Care Ultrasound Method Unveils Potential Imaging Parameters for differentiating Feedlot Respiratory Diseases

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Keywords: Bovine respiratory disease; Feedlot; Interstitial pneumonia; ultrasoun; chute-side.

Abstract

Background: Respiratory disease in cattle is a frequent and important problem. Specific pathological processes vary and interstitial pneumonia (IP) in cattle lacks comprehensive descriptions and understanding. This study aims to use an adapted point-of-care ultrasound (POCUS) method targeting caudo-dorsal region of right lung to determine potential associations between specific imaging parameters at time of treatment and presence of interstitial pneumonia in cattle mortalities. Differentiating between bronchopneumonia and IP could influence treatment strategies and management decisions.

Methods: A cross-sectional study evaluated commercial beef feedlot animals (n=40, 343±13.8 kg; Steer, n=11; Heifer, n=29) chute-side during time of treatment. Only animals that did not recover (mortalities) were included in the study. Chute-side evaluations and necropsies took place from July 10th to July 28th, interstitial pneumonia diagnosis was determined by lung histopathology. Statistical models were generated to evaluate the association of final diagnosis of IP with histopathology findings and imaging parameters. Statistical approach was a stepwise (backward) multivariate logistic regression on interstitial pneumonia outcomes, modeling all the variables collected to identify best fitness model.

Results: Variables significantly (P<0.05) associated with IP diagnosis included B-line count, ultrasound lung score, and A-line count. Sex and days on feed variables were included in final model fixed-effects due to previous associations with IP (P=0.33, P=0.02).

Conclusions: Variables identified with this model show promising potential for chute-side applications. Strategic use of POCUS has showed a potential on differentiating interstitial pneumonias from bronchopneumonia in a timely manner, offering valuable insights for targeted treatment and management decisions in feedlot cattle health.

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Presenter: Vanessa Horton

Swine Chronicles: Unlocking the Secrets of Japanese Encephalitis Virus

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Keywords: Japanese encephalitis virus, rapid systematic review, swine

Abstract

The United States (US) is considered a susceptible region for introduction of the Japanese encephalitis virus (JEV) given available competent mosquito vectors, susceptible hosts (wading birds and pigs) and environmental conditions. As JEV represents a potential threat to the US, ensuring outbreak response preparedness through provision of up-to-date information on disease detection, mechanisms of disease, control and treatment, and impact of disease is crucial. Since pigs are considered the main amplifying host of JEV, a rapid systematic review of the literature on the role of swine (domestic and feral) in JEV epidemiology was conducted. A defined and identical search strategy was used to collect relevant literature from electronic databases (Scopus and Web of Science), as well as important repositories of grey literature. After screening 3,183 abstracts, a total of 224 articles were included for data extraction and synthesis in this review. Transmission of JEV via infected mosquito bites has been widely documented and is well established; however, some evidence of direct oronasal transmission between pigs has been reported. Viremia in infected swine can be detected in as early as one day after infection and persists for approximately 4 to 5 days thereafter. Despite pigs exhibiting a short-lived viremia, JEV has been demonstrated to persist longer in their tonsils. The inability of the porcine host to clear JEV efficiently from this site may explain the important role pigs play as a reservoir and amplifying host. In adult swine, JEV infection may cause reproductive disorders, including delayed farrowing, abortion, stillborn and (or) mummified fetuses, delivery of abnormal litters in sows, and orchitis in boars. Although maternal antibodies can confer protection for up to 4 months of age under field conditions, naïve piglets can manifest neurologic signs including ataxia and tremors between 6 and 21 days after exposure, which may progress to wasting disease or mortality. No specific therapy is currently available for treating JEV infection in pigs. Application of biosecurity practices, vector control, and vaccination are recommended as preventive measures against introduction and spread of JEV in piggeries. Yet, literature supporting the application and effectiveness of biosecurity practices, vector control measures, and risk management practices at the farm level is limited. Although there is no JEV vaccine licensed for pigs in the US, live attenuated vaccines have been reported to elicit superior immunogenicity compared to inactivated vaccines used in endemic countries. Multiple diagnostic tests are available for JEV detection in swine serum or tissues; however, neutralizing anti-JEV antibody titers are considered the gold standard. Although current conditions have not resulted in a JEV incursion in the US, the recent expansion of JEV in Australia highlights the potential for this virus to expand under increasing globalization and climatic change. This rapid systematic review summarized the available literature on the epidemiology, risk factors, pathophysiology, diagnostics, and control measures of JEV in swine populations, to provide a better understanding of this disease and support US preparedness efforts.

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Presenter: Alyssa Leslie

Evaluation of a needle-free injection system for administration of cloprostenol for luteolysis in lactating dairy cows

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Keywords: luteolysis; cloprostenol; needle-free; lactating dairy cows

Abstract

Introduction:Luteolytic drugs are critical for managing reproductive success in dairy cows. The utilization of needles and syringes for administering drugs can result in needle-stick injuries to workers. Needle-free injection systems have been shown to eliminate transmission of bloodborne pathogens in cattle. Data on the efficacy and application of needle-free injection systems in estrous synchronization has not been reported. The objective of the study was to evaluate the effectiveness of a needle-free injection system for intramuscular delivery of cloprostenol to achieve luteolysis in lactating dairy cows.

Methods and Materials: Lactating Holstein dairy cows (n = 26) were randomly assigned to treatment groups utilizing a randomized controlled trial study design. Treatment groups consisted of: 1) needle-free injection of 2 mL (0.5 mg) cloprostenol IM (NFREE-PG; n = 10); 2) traditional needle injection of 2 mL (0.5 mg) cloprostenol IM (NDL-PG; n = 10); 3) needle-free injection of 2 mL physiological saline IM (CNTL; n = 6). All cows were synchronized to achieve an active corpus luteum (CL) at treatment (60 ⍰ 3 DIM). Biomarkers were evaluated 1 h before treatment administration and for 92 h after treatment and included ovarian ultrasound measurements of corpus luteum blood flow and diameter (cm), plasma for cortisol analysis, plasma for progesterone analysis, and plasma for cloprostenol concentration. Data were analyzed using a mixed model with the cow serving as the experimental unit, utilizing commercially available software.

Results: Results with significant effects are presented as least squares means ± SE. There were no differences between the NFREE-PG and NDL-PG groups between 0H to 92H for precent change in CL diameter, however the CNTL group observed a smaller percent change in CL diameter between 0H to 92H (-40.7 +/- 7.3, -39.0 +/- 4.2 vs. 5.8 +/- 8.0, P <0.0001). There were no differences between the NFREE-PG and NDL-PG between 0H to 92H for

percent change in CL volume, however the CNTL observed smaller percent change in CL volume between 0H to 92H (-76.3 +/- 5.2, -72.7 +/- 5.4 vs. 3.2 +/- 10.2, P <0.0001). There were no differences between the NFREE-PG and NDL-PG groups between 0H to 92H for percent change in CL blood flow, however the CNTL observed a smaller change in CL blood flow between 0H to 92H (-93.1 +/- 2.8, -97.1 +/- 2.2 vs. -0.7 +/- 6.2). The NFREE-PG cows had lower cortisol levels (9.8 +/- 1.2 ng/mL) compared to the NDL-PG (17.8 +/- 1.2 ng/mL) and CNTL (19.2 +/- 1.3 ng/mL; P = 0.04).

Conclusion: Similar regression of the CL was observed in both cloprostenol treatment groups. This indicates an effective dose of cloprostenol was administered via the needle-free system. For animals in the CNTL group, CL regression was not observed. These data support the use of a needle-free injection system as an alternative to needle and syringe to deliver cloprostenol for reproductive management in lactating dairy cows. Further research is warranted to evaluate the utilization of needle-free delivery of luteolytic drugs used in estrous synchronization protocols.

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Presenter: Madeline Mancke

Evaluation of cardiac troponin as a predictor of clinical outcome in cattle treated for bovine respiratory disease (BRD) in commercial feedyards

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Keywords: troponin, bovine respiratory disease, diagnostic

Abstract

Intro/Background: Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in feedlot cattle. Prognostic tools would be valuable for managing individual BRD cases in the field. Cardiac troponin (cTn1) is a protein released into the circulatory system after the heart sustains injury. The objective of this study was to determine if there was an association between cTn1 concentrations and clinical outcomes of cattle treated for BRD.

Methods: Blood (approximately 10 mL) was collected from 400 cattle in a central Kansas feedyard at the time of first treatment for BRD. Cattle demographics were also collected for each case. Individual animal treatment outcomes were determined (retreated, died, or railed due to BRD) at 60 days post-enrollment. Troponin concentrations were determined on 318 samples using a commercially-available Bovine Cardiac Troponin ELISA assay.

Results: Of the 318 tested blood samples, troponin concentrations were below the limit of quantification for 310 samples. Eight samples had cTn1 concentrations that were at or above 0.1 ng/mL. There was an association between high cTn1 concentrations and negative cattle outcome.

Conclusions: Cattle with elevated cardiac troponin concentrations at the time of first treatment for BRD were more likely to have negative clinical outcomes. However, low troponin concentrations were not always predictive of treatment success. Failure to predict negative outcomes may be related to stage of BRD at the time of first diagnosis and/or limits with the ability of the troponin assay to detect low concentrations of the protein.

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Presenter: Taylor McAtee

Quantifying the Impact of Feedlot Cattle Performance on Greenhouse Gas Emissions

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Keywords: beef cattle, efficiency, feedlot, greenhouse gas emissions, sustainability

Abstract

In response to the escalating emphasis on sustainability within animal agriculture, stakeholders are increasingly incorporating sustainability terms and goals. This surge in awareness highlights the imperative to assess and measure the impacts of these endeavors. Our study aims to fill this gap by evaluating the associations between performance indicators and the estimated greenhouse gas emissions metric in feedlot cattle cohorts. We used retrospective operational animal health and feedlot performance data from nine U.S. feedlots, encompassing 9,436 cohorts (representing 1,103,761 cattle shipped) our investigation spans the years 2017 to 2021. The primary outcome of interest is the kg of carbon dioxide equivalent emissions per kg of final bodyweight, derived from a proprietary life cycle assessment system that estimates carbon equivalents from predicted greenhouse gas production. Employing a robust mixed effects multivariable regression model, incorporating random effects for feedlot and year, we identified significant associations (P < 0.05) between key performance indicators—namely average daily gain, feed efficiency, and weight—and the emission outcome. Arrival weight of the cohort, sex, and season and a three-way interaction (and corresponding two-way interactions) among arrival weight, sex, and month were significantly (P < 0.05) associated with the outcome. Our results unveiled a noteworthy trend: as average daily gain and feed efficiency increased within the cohort, emissions demonstrated a corresponding decrease. However, these effects were modified by both the sex of the cohort and the season of feedlot arrival. While our findings quantify the effects of performance indicators on greenhouse gas emissions, a gap remains in practical insights, particularly in the context of feedlot cattle. This gap poses a significant challenge, limiting our ability to make well-informed decisions about sustainability in beef production. It is evident from our study that fostering the health and efficiency of cattle in the feedlot is linked to lower greenhouse gas emissions, establishing a foundation for overall sustainability from an emissions perspective.

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Presenter: Megan McGraw

Effect of Seasonal Prescribed Burning on Muscid Fly Populations on Cattle Gazed in the Kansas Flint Hills

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Keywords: Entomology; Horn fly; Prescribed Burning; Arthropod control

Abstract

Horn flies, Hematobia irritans irritans (L.), are common blood-feeding ectoparasites that impact cattle health and wellbeing. Blood feeding can result in significant blood-loss, pain and irritation, culminating in reduced production outputs. Historically, prescribed burning has been used for weed suppression, improving forage quality and pest arthropod control throughout the Midwest. Prescribed burning is traditionally preformed seasonally in either the spring, summer, or fall. While the season of burning has been shown to effect plant growth, little is known of the impact on pest arthropods like horn flies. This study aimed to determine if horn fly populations are impacted by the season of which the burning is performed. The Kansas State University Beef Stocker Unit is a 1,000 acre facility consisting of 16 pastures with each being assigned to one burn season (spring, summer or fall). Pastures have been burned consistently in their designated season for the past four years and are stocked equally at 246 pound per acre. Starting two weeks after the cattle were released onto pasture, a long-range DSL camera was used to take photographs of different steers in each pasture for 11 weeks. ImageJ software was used to manually count flies on each of the photographs over the study period. Initial results suggest that burn season does impact the number of horn flies with cattle on summer burned pastures having the highest fly burdens. Understanding the impact of burn timing on arthropod pest burdens can be used by producers to make informed decisions on burn treatment options.

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Presenter: Trey Neyland

The Effect of Flunixin Meglumine on Viral Shedding in Calves

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Keywords: Non-steroidal anti-inflammatories; bovine herpesvirus-1; flunixin meglumine; viral; shedding

Abstract

Background: Non-steroidal anti-inflammatories (NSAIDs) are a common treatment for pyrexia in cattle with bovine respiratory disease (BRD). NSAIDs have known immunomodulatory effects in cattle with BRD. Minimal research has evaluated the effects that Food and Drug Administration approved NSAIDs have on viral shedding in clinical BRD cattle.

Hypothesis/Objective: The hypothesis of the study was that flunixin meglumine would increase the magnitude and duration of viral shedding in calves inoculated with bovine herpesvirus-1 (BHV-1). Also, to investigate the shedding characteristics of BHV-1.

Animals: Twelve Holstein cross-bred steer calves equally randomized into treatment (FM) group and control (CON) groups.

Methods: All calves were inoculated intranasally with approximately 4-mLs of 1x10^5 TCID50 of BHV-1. Nasal swabs for BHV-1 PCR testing were collected every 24 hours for a minimum of 7 samples post-initial positive. Calves in the FM group were treated with 2.2-mg/kg of flunixin meglumine intravenously after the first BHV-1 PCR positive sample. PCR sampling for a calf ceased after 2 consecutive negative PCR samples.

Results: Shedding of BHV-1 ranged from 1 to 17 days for animals in both FM and CON groups. There was no statistical difference in magnitude or duration of BHV-1 shedding between groups.

Conclusions and Clinical Importance: Within the study, intranasal inoculation with BHV-1 resulted in a wide range of shedding magnitude and duration. Flunixin meglumine administration in BHV-1 challenged calves did not result in an increase in magnitude or duration of viral shedding as analyzed.

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Presenter: Konner Cool

Ferrets as a Model for SARS-CoV-2 Infection During Pregnancy

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Keywords: COVID-19, SARS-CoV-2, Pregnancy, Ferrets

Abstract

The emergence of SARS-CoV-2 and subsequent COVID-19 pandemic in humans created an urgent need for the development of animal models to evaluate therapeutic and preventative mitigation strategies. Historically, ferrets (Mustela putorius furo) have been a reliable model for studying the clinical and pathophysiological aspects of respiratory pathogens in humans. The susceptibility of ferrets to SARS-CoV-2 has been thoroughly documented and provides a reasonable model for sub-clinical SARS-CoV-2 infection in humans. The goal of this study was to

further develop the ferret model for use in evaluating SARS-CoV-2 infection and its effect on pregnancy. Eight late-term pregnant (jills) and eight spayed (sprites) ferrets were enrolled in this study and monitored daily for clinical signs, temperature, and weight changes. Six jills and six sprites were challenged intranasally and orally with the SARS-CoV-2 Alpha variant of concern (VOC) USA/CA-5574/2020 (B.1.1.7). Clinical samples were collected at regular intervals and evaluated for SARS-CoV-2 RNA and infectious virus as well as SARS-CoV-2 specific antibody responses and virus-specific lesions. Post-mortem examinations and tissue collections were conducted at 4- and 7- days post challenge (DPC). Fetuses were collected from the 6 infected jills (n= 48 total; 29 at 4 DPC, 19 at 7 DPC) and 2 negative control jills (n=18 total; 9 at 3 DPC, 9 at 5 DPC). Generally, pregnant jills had higher levels of SARS-CoV-2 RNA and infectious virus shed via the nasal and oral cavities than sprites. Likewise, higher loads of SARS-CoV-2 RNA and infectious virus were recovered from the respiratory and lymphoid tissues of pregnant jills at 4 and 7 DPC. Similar quantities and distribution of SARS-CoV-2 RNA were observed in other visceral tissues between the two groups. Importantly, two fetuses from one jill (2/48 fetuses total) at 4 DPC had low levels of SARS-CoV-2 RNA. This data is consistent with reports of more severe COVID-19 disease in late-term human pregnancies and rare reports of vertical transmission in humans. Further development of this model should evaluate maternal and fetal immune responses as well as host and viral factors that may be responsible for the severe disease outcomes observed in humans. This data demonstrates that the COVID-19 ferret model could be used to investigate the safety and efficacy of therapeutic interventions throughout gestation.

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Presenter: Douglas Farleigh

Preliminary analysis of canine cancer cases and environmental contaminants in Kansas (2021-2022)

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Keywords: Canines; Superfund; sentinel animals; carcinogens

Abstract

The carcinogenicity of various environmental contaminants in humans has been studied extensively in recent years. However, evaluations of carcinogenicity of environmental contaminants in canines and the utility of the domestic canine as a sentinel for carcinogen exposure in the literature are scant. The present study was to investigate these knowledge gaps and evaluate the relationship between canine cancer occurrence and environmental contaminants from Superfund sites. Data on diagnoses of carcinomas, sarcomas, and mast cell tumors in dogs from years 2021-2022 were collected from the Kansas State Veterinary Diagnostic Laboratory (n = 2,803, 39% carcinomas, 21% sarcomas, 40% mast cell tumors). The 3 most reported breeds were the Labrador Retriever, Boxer, and Pit Bull Terrier. The median age was 9 years old (range 0-24 years). The cases were sorted and normalized to human population per ZIP code to reduce reporting bias and identify the top 5 hot spots of reported canine cancer cases. Location data on Superfund sites (n = 14) in the state of Kansas were collected from the Environmental Protection Agency's Superfund National Priorities List. Superfund sites are locations under the jurisdiction of the "Superfund" Act, formally known as the Comprehensive Environmental Response, Compensation and Liability Act, which are known to be contaminated with hazardous waste posing risks to human health, animal health, and the environment. Many of these sites are contaminated with known carcinogens, such as organochlorine pesticides, dichlorodiphenyltrichloroethane, and volatile organic compounds; several studies have demonstrated the domestic canine's potential as a sentinel for human and environmental health by demonstrating development of similar diseases in humans and canines following exposure to various contaminants. Exploratory hotspot analysis was conducted to investigate if reported cases were clustered spatially. Five hotspot ZIP codes were identified. To further investigate canine cancers, data on Superfund sites were gathered for Kansas and displayed on the map. Buffer zone analysis of the canine cancer case data revealed a total of 684 cases within 5 miles of at least one of the 14 Superfund sites in Kansas. Of these, 275 cases were within 3 miles and 409 cases were between 3 and 5 miles of at least one of the 14 Superfund sites. The preliminary analysis justifies investigation of the relationship between the distance from a Superfund site and occurrence of canine cancers in the state of Kansas.

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Presenter: Chandler Hansen

Assessing Veterinarian's Perspectives on the Applicability of Anatomy Education to Clinical Practice

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Keywords: anatomy; education; survey; CBVE

Abstract

Introduction: Veterinary anatomy forms a foundational knowledge for veterinarians, enabling them to understand the structure and function of animals' bodies. The quality and effectiveness of anatomy education in veterinary school plays a crucial role in preparing graduates for successful careers. However, there's a gap in understanding how well veterinary anatomy courses align with the practical needs of veterinary professionals. Competency-based education has become the preferred model in healthcare education, with many veterinary schools adopting the principles of competency-based veterinary education (CBVE). As instructors to first-year veterinary students, we are distanced from clinical practice. This study aims to assess the effectiveness of the anatomy courses at the Kansas State University College of Veterinary Medicine in preparing graduates for their professional careers. By gathering real-world perceptions, the KSU CVM anatomy team can ensure that the curriculum aligns effectively with the competencies required in contemporary veterinary practice. This study seeks to facilitate continuous improvement by seeking alumni perceptions that will assist in adapting anatomy teaching strategies to align with CBVE outcomes and meet the evolving needs of the profession.

Methods: In a pilot study, a brief Qualtrics survey was distributed to KSU CVM alumni from the graduating Class of 2023. The survey included 15 questions designed to elicit retrospective opinions from clinicians about the adequacy of the anatomy curriculum in preparing them for their veterinary careers, the usefulness of anatomy in daily practice, and suggestions for improvement. Participation was voluntary, and responses were collected anonymously to encourage honest feedback.

Results: Preliminary analysis of the pilot study data revealed positive perceptions of KSU CVM anatomy courses, with comments and suggestions outlining how our courses can be improved. 61% of respondents have a small animal focus, with 22% practicing mixed animal medicine. 61% of respondents indicated that the knowledge gained from KSU CVM anatomy courses has been very or extremely applicable to their careers. 61% of respondents also stated that KSU CVM anatomy education has contributed to their success in their veterinary career. 50% of respondents sometimes or never recall the anatomical name of muscles, blood vessels, nerves, etc.

A thematic analysis of open-ended responses revealed requests for more clinical integration through relation of anatomical structures to real-world scenarios. Additionally, respondents suggest discussing surgical approaches during the course to foster critical thinking associated with practical applications.

Conclusions: In summary, our pilot study revealed overall positive perceptions among alumni regarding the applicability of KSU CVM anatomy courses. However, the study identifies opportunities for improvement, particularly in enhancing clinical relevance. Respondents emphasize the need for discussing surgical approaches during the course, highlighting the importance of aligning anatomical education with clinical veterinary practice and the CBVE framework. Next steps include refining the survey questions to be sent to a broader population of KSU CVM alumni. The opinions of our alumni will guide course refinements, ensuring our graduates are well-equipped for the dynamic challenges of their veterinary careers.

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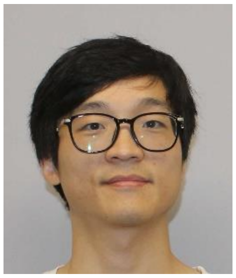

Presenter: Dongoh Lee

Enhancement of chloride versus bicarbonate conductance by CFTR modulators

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Keywords: ion channel; CFTR; trikafta; bicarbonate; chloride

Abstract

Introduction: Cystic fibrosis (CF) is a life-shortening multiorgan disease that is associated with variant forms of the cystic fibrosis transmembrane conductance regulator (CFTR) that exhibit reduced epithelial chloride and/or bicarbonate permeation leading, in the airway, to luminal mucus build-up and recurrent bacterial infections. Potentiators (e.g., VX770), which enhance CFTR channel gating to increase open probability, and correctors (e.g., VX445 and VX661), which modulate trafficking and maturation to improve CFTR expression in the cell membrane, were discovered and optimized using assays that assessed Cl- secretion. However, bicarbonate permeation has not been described for CFTR variants in the absence or presence of these clinically important drugs.

Methods: Total forskolin-stimulated conductance and the relative bicarbonate-to-chloride conductance (gHCO3/Cl) were measured in CFTR variants expressed in Fisher rat thyroid (FRT) cells using an anion substitution approach.

Results: The gHCO3/Cl of wild type CFTR induced by forskolin was similar to previous literature ~ 0.1. Subsequent exposure to VX770 enhanced total conductance while having no detectable effect on gHCO3/Cl. Pre-treatment of either or both correctors modestly increased overall conductance and gHCO3/Cl was elevated following overnight exposure to VX445, alone, or combined with VX661. Cells expressing the most common clinically-important CFTR variant, F508del CFTR, exhibited minimally detectable responses to forskolin and VX770. However, following pre-treatment with correctors, VX445 and/or VX661, forskolin-induced conductance was observed and was potentiated by VX770. Importantly, the gHCO3/Cl of F508del CFTR is negligible in all conditions. Conductance of a second clinically important variant, G551D CFTR, showed little response to forskolin, but substantial potentiation by VX770. The gHCO3/Cl was minimal following exposure to VX770, but was enhanced by pre-incubation with VX445 alone or combined with VX661. Additionally, we observed conductance induced by acute exposure to VX445, demonstrating a role as potentiator as well as corrector for G551D CFTR, while exhibiting only corrector effects on wild-type CFTR. Concurrent experiments showed enhanced conductance in all CFTR variants as pH increased from 6.5 to 8.5, which may indicate a positive feedback mechanism mediated by CFTR-dependent bicarbonate secretion.

Conclusions: Tissues most affected in CF (e.g., pancreas, intestine, male reproductive tract) are known to secrete substantial amounts of bicarbonate. Correctors and potentiators enhance Cl- conductance and greatly enhance the health and quality of life for most CF patients. However, the absence of detectable bicarbonate conductance by F508del CFTR in all conditions and G551D CFTR in the absence of correctors may reveal new types of symptomology associated with extracellular pH regulation as patients are maintained on these drugs for extended periods.

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Presenter: Daniel Madden

Immune dysregulation at the cellular, cytokine, and transcriptomic level during African swine fever virus infection

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Keywords: African swine fever; cytokines; innate immunity; cellular immunity; transcriptomics

Abstract

INTRODUCTION: Overwhelming pro-inflammatory responses are considered a primary pathogenic mechanism of African swine fever virus (ASFV) infection. To clarify the role of host immune responses in the pathogenesis of African swine fever (ASF), we sought to characterize innate and early adaptive immune responses to lethal ASFV infection and assess the temporal dynamics of major changes in immune parameters in relation to clinical progression of acute ASF.

METHODS: Blood and plasma were serially collected from six pigs infected with a genotype II ASFV strain (Armenia 2007) prior to ASFV inoculation and at 1, 3, 5, and 7 days post-challenge (DPC). An array of pro- and anti-inflammatory cytokines were quantified in plasma using capture ELISA. Circulating immune cells were phenotyped using swine-specific flow cytometry antibody panels to evaluate changes in circulating lymphoid and myeloid cell populations. RNA-Seq was performed on whole blood RNA extracts using the Illumina NextSeq platform. Differentially expressed genes (DEGs) were identified for each timepoint compared to pre-challenge control using DESeq2, and gene ontology (GO) pathway enrichment was evaluated using the PANTHER software system.

RESULTS: Following ASFV challenge, rapid increases in pro-inflammatory cytokines including type I interferons, IL-12p40, and TNF-α were observed at 5 and 7 DPC, coinciding with the onset of severe clinical disease and high viral DNA loads. Levels of the anti-inflammatory cytokine IL-10 were elevated at 5- and 7- days post-challenge (DPC), while concentrations of the immunoregulatory cytokine TGF-β1 decreased over time. Lymphopenia characterized by decreases in B cell, CD8+ T cell, and NK cell proportions was observed, while the level of CD4+ T cells remained stable and the percentage of CD4+CD8+ T cells increased over time. Significant fluctuations in circulating monocyte and macrophage levels were observed throughout infection, most notably an abrupt spike in CD203+ mature macrophages immediately prior to death. The number of DEGs found in whole blood increased over time, peaking at 7 DPC. Pathways associated with cytoplasmic translation and ribosome formation were downregulated at 1 DPC and 5 DPC, while processes involving innate immunity and host defenses against viral pathogens were strongly upregulated at 5 DPC and 7 DPC.

CONCLUSIONS: Lethal ASFV infection produces an immunological state characterized by large increases in circulating pro-inflammatory cytokines, inadequate anti-inflammatory cytokine responses, progressive lymphopenia, shifting monocyte/macrophage populations and phenotypes, and transcriptional activation of multiple host immune pathways. These results highlight the pro-inflammatory immune responses in acute ASF and can inform research into novel antiviral and vaccine development strategies for ASF.

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Presenter: Chester D McDowell

Development of long-read targeted whole genome sequencing for African and classical swine fever viruses.

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Keywords: African swine fever virus; Classical swine fever virus; MinION sequencing; Transboundary animal diseases

Abstract

Introduction: African swine fever virus (ASFV) and classical swine fever virus (CSFV) are important transboundary animal diseases (TADs) affecting swine. ASFV is a large DNA virus with a genome size of 170-190 kilobases (kB) belonging to the family Asfarviridae, genus Asfivirus. CSFV is an RNA virus with a genome size of approximately 12 kB belonging to the family Flaviviridae, genus Pestivirus. Outbreaks involving either one of these viruses can result in massive culling of swine and export restrictions of pork products, leading to significant economic losses to the pork industry in affected countries. Current detection methods during an outbreak provide minimal genetic information on the circulating virus strains and genotypes. Due to the increasing availability and reduced cost of

next generation sequencing (NGS), it is vital to have NGS protocols in place for the rapid identification and genetic characterization of ASFV and CSFV for outbreak and surveillance situations.

Methods: In this study, panels of primers spanning the genomes of ASFV or CSFV were developed to generate approximately 10kB (ASFV) or 6kB (CSFV) amplicons. Primer panels consisting of 19 primer pairs for ASFV and 2 primer pairs for CSFV, provide whole genome amplification of each pathogen. These primer pairs were further optimized for batch primer pooling and thermocycling conditions. Following the optimization of primer pools, a total of 5 primer pools/reactions were used for ASFV and 2 primer pairs/reactions for CSFV. The ASFV primer pools were tested on viral DNA extracted from clinical samples collected from pigs experimentally infected with ASFV genotype II viruses. The CSFV primer panel was tested on viral RNA extracted from 9 different strains of CSFV representing the 3 known CSFV genotypes and 21 clinical samples collected from pigs experimentally infected with 2 different genotype 1 CSFV strains. ASFV and CSFV amplicons from optimized PCR/RT-PCR reactions were subsequently sequenced on the Oxford Nanopore MinION platform.

Results: The targeted protocols for amplification and sequencing of ASFV and CSFVresulted in an average coverage greater than 1000X for ASFV with 99% of the genome covered, and an average coverage of 10,000X to 20,000X for CSFV with 97% to 99% of the genome covered. The ASFV targeted whole genome sequencing protocol has been optimized for genotype II ASFVs which have been involved in recent outbreaks; optimization for other ASFV genotypes is in progress. The CSFV targeted whole genome sequencing protocol has potential universal applications for the detection of all currently known CSFV genotypes.

Conclusions: The established targeted amplification and NGS protocols for ASFV and CSFV will be important tools to assist in early pathogen detection and genomic characterization of these high consequence swine viruses in outbreak and surveillance situations globally and within the United States, should an outbreak occur.

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Presenter: Theresa A. Quintana

Characterization of ABC transporter genes in Toxocara canis using RNA-Seq.

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Keywords: canine, parasite, zoonotic, RNA-seq.

Abstract

Background: Toxocara canis (canine roundworm) is a ubiquitous canine nematode that presents risks to human health. Eradication is complicated by the ability of T. canis third-stage larvae to undergo developmental arrest in the host somatic tissue. Macrocyclic lactone anthelmintics do not kill somatic larvae, despite their adequate distribution throughout the host's body.

Objective and Hypothesis: Our study aimed to investigate the differential expression of mRNA transcripts in T. canis third-stage larvae treated with ivermectin (IVM) or moxidectin (MOX) compared to untreated controls to identify up- or downregulated genes, with particular interest in ABC transporter genes. Our working hypothesis is that ivermectin (IVM) and/or moxidectin (MOX) induce transcriptional changes among ABC transporter genes in third-stage infective larvae. Several gaps exist in our understanding of their role in anthelmintic tolerance. Our central hypothesis is that ATP-binding cassette (ABC) transporters, such as P-glycoproteins (Pgps), play a role in drug tolerance via the efflux of anthelmintic compounds.

Methods: In this study, we isolated eggs from the uterus of adult T. canis female worms and incubated the eggs at 25°C to allow development to third-stage larvae. Using a chemical hatch protocol, we isolated larvae (n = 500 each) and subjected them to three conditions: incubation with IVM, MOX, or no treatment (controls), in biological triplicates. Adult male and female worms were also used as controls. We isolated RNA from each sample with Trizol and quantified it with Qubit. We then prepared libraries using an Illumina Stranded mRNA prep kit and sequenced it on a NextSeq sequencer. We analyzed the data using the HISAT2-featurecounts and RNA STAR pipelines. Using DESeq2, we quantified the expression of ABC transporter genes and identified differentially expressed transcripts.

Results: Compared to higher eukaryotes, parasitic nematodes have a diverse repertoire of ABC transporter genes. We have identified 62 ABC transporters in the genome of T. canis. In the preliminary analysis, IVM-treated L3s exhibited the upregulation of 27 genes and the downregulation of 17 genes compared to the control group. Similarly, MOX-treated L3s showed the upregulation of 55 genes and the downregulation of 161 genes compared to the controls. Further investigation identified that some ABC transporters were differentially expressed.

Conclusions: Our study highlights the importance of further characterizing these ABC transporters and their role in drug disposition to identify new targets for further investigation. Future research will focus on investigating several ABC genes that were found to be upregulated in treated larvae to determine their functional transport capabilities of macrocyclic lactones. This may aid the development of nematode-specific ABC transport inhibitors that can enhance antiparasitic efficacy against somatic larval reservoirs, potentially mitigating human toxocariasis.

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Presenter: Cameron Sutherland

Evaluation of Environmental Bartonella spp. in Flea-Infested Homes

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Keywords: Bartonella; fleas; environment

Abstract

Introduction: Ctenocephalides felis, known colloquially as the “cat flea” is an ectoparasite of human and veterinary importance. This parasite is responsible for FAD in hosts due to a hypersensitivity reaction as well as vectoring Bartonella spp. Bartonella spp. is a Gram-negative rod responsible for the disease colloquially as “cat scratch fever”.

Methods: Fifty-five homes were enrolled in the West-Central Region of Tampa, Florida based on level of animal and environmental flea burden. Homes were sub-selected into categories including 21 “High” homes (≥ 5 fleas on ≥ 1 cat, ≥ 5 fleas on environmental traps), 24 “Low” homes (< 5 fleas on all cats, < 5 fleas on traps), and 10 “No” homes (0 fleas on all cats, 0 fleas on traps). Samples were collected using a 25 mm Micro-Vac Cassette attached to a Zefon 120v Diaphragm pump by passing the cassette ten times vertically and ten times horizontally within in a 1’x1’ square at two different sections of carpet/flooring in the home. Cassette filters were rinsed using sterile 1% PBS and extracted following the Qiagen DNeasy Blood and Tissue Kit Protocol. Extractions were processed through qPCR testing for Bartonella spp. In addition, oral and nasal swabs were taken from each cat in enrolled homes. All nasal samples were extracted and then PCR was conducted for Bartonella spp.

Results: Of all 55 homes with vacuum samples collected, 12 homes tested positive for Bartonella spp. Of all 55 homes with cat samples collected, 7 homes had at least one cat that tested positive for Bartonella spp. A Fisher’s exact test showed that these two bodies of samples agree (p = 0.0037).

Conclusion: Using a vacuum cassette attachment is a feasible method for detecting Bartonella spp. in the environment. In addition, the cat oral/mouth swabs are sampling from the same environment as the vacuum samples as they showed statistical agreement.

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Presenter: Alexandria Zabiegala

Comparative analysis of various host proteases in the entry of SARS-CoV-2, SARS-CoV, and MERS-CoV using the pseudovirus assay

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Keywords: Coronaviruses; Entry; Spike Protein; Host Proteases

Abstract

INTRODUCTION: Host proteases, specifically furin and transmembrane serene protease 2 (TMPRSS2), have been shown to directly influence the entry of coronaviruses through processing of the spike protein (S). Coronavirus S protein contains two major cleavage sites: S1/S2 junction, which is variable among coronaviruses, and S2’, which is highly conserved among coronaviruses. For SARS-CoV-2, SARS-CoV, and MERS-CoV, TMPRSS2 has been shown to cleave S2’, subsequently exposing the fusion domain and allowing for direct fusion of the viral envelope to the cell membrane, facilitating efficient entry. In the absence of TMPRSS2, viruses enter through a less efficient endosomal route. While the effect of TMPRSS2 in the entry of these coronaviruses has been studied well, the processing potential of other host membrane proteases is poorly understood. In our study, we assessed the effect of the expression of human airway trypsin-like protease (HAT), matriptase-2 (TMPRSS6), and corin on entry of pseudoviruses expressing S of SARS-CoV-2, SARS-CoV, or MERS-CoV.

METHODS: We generated lentiviral-based pseudoviruses expressing S from SARS-CoV-2, SARS-CoV, or MERS-CoV and carrying the firefly luciferase gene. HEK293T cells that are expressing ACE2, the functional receptor of SARS-CoV-2 and SARS-CoV, or DPP4, the functional receptor of MERS-CoV, with or without TMPRSS2, HAT, matriptase-2, or corin were generated by transfecting the cells with a plasmid encoding each receptor gene. To assess the roles of the cellular protease in virus entry, cells were transduced with pseudoviruses, and entry of pseudoviruses was measured using a dual luciferase assay reporter system.

RESULTS: We found that the presence of both TMPRSS2 and a functional receptor significantly increased entry of SARS-CoV-2, SARS-CoV, and MERS-CoV pseudoviruses by 43-, 7- and 67-folds, respectively, compared to those in cells expressing only TMPRSS2. Expression of matriptase-2 with a functional receptor also significantly increased the entry of all pseudoviruses when compared to those in cells expressing a functional receptor alone. However, HAT or corin expression with a functional receptor did not show any significant effect in the entry of all pseudoviruses compared to those in cells expressing a functional receptor alone.

CONCLUSIONS: We demonstrated that co-expression of TMPRSS2 or matriptase-2, but not HAT or corin, with a functional receptor in cells significantly increased the entry of all pseudoviruses at varying levels. These studies suggest that multiple proteases may be involved in the entry of these highly pathogenic human coronaviruses, which may contribute to the comparative pathogenicity.

Presenter: Danielle Lopez

Can Anxiety Fog Your Memory? An Alzheimer’s Disease Animal Model

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Keywords: Alzheimer's Disease, Rodent Model, Anxiety, Elevated Plus Maze

Abstract

Alzheimer’s disease (AD) is characterized by cognitive impairments, however, heightened anxiety often accompanies and in some cases exacerbates cognitive its. The present study aims to understand the influence of multiple variables on anxiety-like behavior in TgF344-AD rats and determine if anxiety impacts memory performance. METHODS: An elevated plus maze was used to assess anxiety-like behavior in the established colony (n=107). Influences of age, sex, genotype, and exercise on anxiety were evaluated via multiple linear regression. One-way ANOVA was used to identify between-group differences in anxiety behavior. Correlation analysis evaluated the relationship between anxiety and memory performance. RESULTS: Age (p<0.05) and AD genotype (p<0.001) were associated with increasing anxiety, while exercise (p<0.05) was associated with decreasing anxiety. Female AD animals displayed more anxiety-like behavior versus WT female (p<0.001) and AD male (p<0.05) littermates. Correlation analysis revealed no relationship between anxiety behavior and memory performance. DISCUSSION: Concluding that while factors such as age, sex, AD genotype, and training status can impact anxiety levels in the TgF344-AD model, anxiety level did not impact memory performance.

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Presenter: Deanna Gennett

Evaluating estimated carbon emissions and feedlot production data to address beef sustainability

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Keywords: Beef, Sustainability, Emissions

Abstract

OBJECTIVE: With climate change and agricultural sustainability considered major societal issues, the goal of this study was to provide insight on how feedlot cattle, directly and indirectly, affect greenhouse gas emissions, and to demonstrate relationships between feedlot cattle health, performance, and greenhouse gas emissions. The specific objective of this study was to determine how various feedlot metrics, including for cattle health and performance, are associated with estimates of greenhouse gas emissions.

METHODS: Operational animal health and feedlot performance data were obtained from multiple commercial feedlots via formal data sharing agreements. These data were linked by unique lot (cohort) identifiers with outputs from a proprietary life cycle assessment system that estimates carbon equivalents from predicted greenhouse gas production. The primary dataset included over 10,000 lots (cohorts) of cattle containing a range of 10-358 animals per lot. Descriptive graphs and figures were created from the cattle health and performance metrics, and the corresponding lot-level emissions estimates for data analysis, visualization, and pattern identification.

RESULTS: Using the estimated emissions, the cow-calf, or pre-feedlot, phase of production was associated with approximately 80% of a cattle's total lifetime emissions while the feedlot sector was associated with the remaining 20%. Our data indicated that the production and delivery of feed (49%) comprises the largest source of greenhouse gas production during a cattle's life cycle with the remaining coming from enteric fermentation (27%), manure (21%), and fuel (3%). Within their life cycle, steers produced 4.47% more emissions than heifers on a per animal basis, while heifers produced 7.39% more emissions on a per kilogram of live cattle weight basis (as a measure of beef production). Lifetime emissions per kilogram of cattle weight were reduced when days on feed in the feedlot and mortality were minimal and weight gain relative to feed intake was high.

CONCLUSIONS: Based on this study and other ongoing work, we can conclude that cattle health and performance in the feedlot have an impact on the quantity of greenhouse gas emissions that are generated during beef production. In other words, healthy and efficient cattle in the feedlot are associated with less greenhouse gas emissions and are overall more sustainable from an emissions perspective. Recognizing how cattle production systems impact carbon emissions is a first step towards improving beef sustainability and reducing the associated carbon footprint.

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Presenter: Elayna Anderson

Can Florfenicol be a Substitute for Chloramphenicol in Resistant Canine UTIs?

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Keywords: canine; excretion; florfenicol; antimicrobial stewardship; urinary tract infection

Abstract

The urinary excretion profile of florfenicol was evaluated in dogs to explore its potential for treating canine urinary tract infections. Nine healthy male intact purpose-bred Beagles and four healthy client-owned dogs each received a single oral dose of florfenicol 20mg/kg (300mg/ml parenteral solution) with food. All voluntary urinations were collected for 12 hours. Urine was measured for volume and florfenicol concentration using high performance liquid chromatography. Although florfenicol is reportedly bitter tasting, 7/9 Beagles and 4/4 client-owned dogs completely ingested the florfenicol and were enrolled; salivation (n=1) and head-shaking (n=3) were observed. The last measurable urine florfenicol concentrations were variable: Beagles (0.23-3.19 mcg/mL), Pug (3.01 mcg/mL), English Setter (21.29 mcg/mL), Greyhound (32.68 mcg/mL), and Standard Poodle (13.00 mcg/mL). Urine half-life was similar for the Beagles and the Pug, 0.75-1.39 h, whereas the half-life was 1.70-1.82 h for the English Setter, Greyhound, and Standard Poodle. Larger breed dogs exceeded 8 mcg/mL florfenicol (wild-type cutoff) in their urine at 12 h, whereas the Beagles and Pug had <8 mcg/mL; it is unclear if this is an individual, breed or size difference. These data suggest oral florfenicol may need to be administered q6-12h for canine urinary tract infections, but further data are needed (more enrolled dogs, multiple dose regimens) before considering clinical trials or breed specific differences.

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Presenter: Gabrielle Maroulis

Observational Assessment of Water Use in a Large Animal Teaching Hospital

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Keywords: Sustainability; Veterinary medicine; Water conservation

Abstract

Water use in healthcare facilities comprise approximately 7% of total water use in the U.S. commercial sector, accounting for up to 350 gallons per hospital bed per day. There is a paucity of data surrounding this topic in veterinary medicine. Large animal hospitals require frequent use of water for proper sanitation of patient-occupied areas (e.g., stalls, trafficked walkways, cattle-chutes). We hypothesized large animal teaching hospital (LATH) water use, for sanitation purposes only (i.e., hoses and sinks), would comprise more than 10% of the total water entering the building complex encompassing the LATH. Additionally, water use would trend positively with the number of patients seen. A commercially available flow meter was installed on seventy-three hoses and twenty-one faucets in the LATH. The volume of water (gallons/week) consumed each week was monitored a single time and is currently ongoing. Total water volume measured over the course of this investigation (31 weeks) totaled 475,192.4 gallons, costing $5,664.29. Patient areas were responsible for 84% of water measured, accounting for 201 gal/patient, costing $3.42 patient/day. Perioperative rooms accounted for 14% of water use. The LATH water use comprised 14.1% of the building complex’s water. These observations provide objective data on water use for a single LATH and will be used to compare water conservation strategies. One method for water conversation was identified as reducing the flow (gallons per minute) for hoses in the LATH. The average flow was 7.2 gallons per minute (gpm). At the conclusion of week 31 of data collection, 5 gpm flow restrictors were installed on fifty hoses in the LATH. The volume of water consumed was monitored a single time and is currently ongoing. Further investigation on how this data may be harnessed for water conservation strategies, benefiting environmental and financial goals is warranted.

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Presenter: Greta Karwath

Pharmacokinetic comparison and bioequivalence of emodepside formulations in dogs

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Keywords: pharmacology; hookworm; canine; bioequivalence; pharmacokinetic

Abstract

Emodepside, administered as the European Medicines Agency-approved canine oral formulation, has been found to be the lone effective treatment for multi-drug resistant (MDR) Ancylostoma caninum (dog hookworm) infections in dogs. In the United States, emodepside is only FDA-approved as a topical product for cats. Veterinarians in the United State have begun administrating the feline topical product orally in an extra-label manner to dogs with MDR hookworm infections despite the unknown pharmacokinetics. Additionally, no studies have been undertaken to determine if the feline topical formulation administered orally results in the same blood concentrations as the effective canine oral formulation (bioequivalence) to determine if both formulations would be expected to yield equivalent effects. Using a crossover design, the topical feline and canine oral emodepside formulations were administered to 7 client-owned dogs. Following drug administration, serial blood samples were taken over a 21-day period. Using liquid chromatography coupled with mass spectrometry, emodepside was quantified in plasma. Non-compartmental pharmacokinetic analysis and bioequivalence testing were carried out using commercially available software. The results of this study are pending but will be utilized to inform the treatment of MDR hookworm infections in dogs

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Presenter: Grace Schieferecke

Molecular Analysis of Benzimidazole Resistance in Hookworms in Kansas Dogs

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Keywords: canine zoonotic hookworm resistance

Abstract

Ancylostoma caninum (canine hookworm) primarily causes infections in dogs, but can also be zoonotic. Hookworm infections are the most commonly diagnosed parasites in dogs in the U.S., with up to 4% prevalence. Common treatment for hookworm infections includes a class of antiparasitics called benzimidazoles that hookworms have developed resistance to. A single nucleotide polymorphism (SNP) in codon 167 (F->Y) of the beta-tubulin gene is suggested to be responsible for benzimidazole resistance and is therefore a target for resistance diagnosis. There is little research on this SNP in the U.S., particularly in Kansas. We hypothesize that this mutation is prevalent in hookworms in Kansas dogs. We isolated hookworm eggs from the feces of naturally infected dogs and extracted DNA using a commercial kit. Using qAC167 and qACRtr1 primers, we performed quantitative polymerase chain reaction (qPCR) on ~80 samples to determine relative allele frequency rates at codon 167. We found significant variation in allele frequencies within and between samples, with variations from 1% to nearly 100%. These results are significant, novel, and have shown the existence of significant resistance within A. caninum in the region. These have implications for the judicious use of benzimidazoles for treating hookworm infections in dogs without contributing to more resistance development. By using molecular techniques, we demonstrate that we can detect resistance early and in individual dogs. These results can be integral for choosing an effective treatment for hookworm infections. Molecular diagnostics are key for treating infections with probable levels of resistance.

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Presenter: Haitham Alneaemy

Lysine and lactate, alone or in combination, as substrates for *Fusobacterium necrophorum* in an in vitro ruminal fermentation system

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Keywords: Liver abscess, *Fusobacterium necrophorum*, Lysine, Lacate, In vitro rumen fermentation system

Abstract

Liver abscesses generally occur in finishing cattle fed high-grain, low-roughage diets and have a significant economic impact on the feedlot industry. Cattle with abscessed livers seldom show any clinical signs and are detected only at slaughter. Liver abscesses comprise, on average, 67% of all liver abnormalities in cattle slaughtered in the United States with a prevalence of 10-20% and may reduce the value of the beef carcass up to $38 per animal with the most severe abscesses. The main etiologic agents of liver abscesses are *Fusobacterium necrophorum* subsp. *necrophorum* and F. *necrophorum* subsp. *funduliforme*. *Fusobacterium necrophorum*, a Gram-negative anaerobic ruminal bacterium, is generally higher in grain-fed than forage-fed cattle. Fusobacterium utilizes lysine and lactate as an energy source for growth. Our objective was to investigate lysine and lactate alone or in combination on the growth of Fusobacterium in a rumen simulating in vitro fermentation system. *Fusobacterium necrophorum* strains previously isolated from liver abscesses of feedlot cattle were used. Rumen fluid was collected from a ruminally-cannulated steer, strained through four layers of cheese cloth, and diluted 1:1 with McDougall's buffer and dispensed in 100 ml volumes into serum bottles. The pH of the rumen fluid was recorded. Lysine and lactate alone or in combination were added at 0 (control) or 100 mM. *Fusobacterium necrophorum* strains (both subspecies) were inoculated at two different concentrations (1 x 10^7 CFU/ml at 0.1 ml or 1 ml). Serum bottle was flushed with CO2, sealed with rubber stoppers and incubated at 39°C. The serum bottles were sampled at 0, 12, and 24 h and pH were recorded. DNA was isolated by boil prep method and purified using a GeneClean turbo kit as per the manufacturer's recommendation. A real-time qPCR assay targeting the subspecies-specific promoter sequences of the leukotoxin gene, *lktA*, was employed to detect and quantify *F. necrophorum*. The experiment was conducted in duplicates for each treatment and repeated thrice. Statistical analysis was done using PROC MIXED and GLIMMIX procedures (SAS v. 9.4). Lysine and lactate alone or in combination had higher growth of both subspecies of Fusobacterium at 12 or 24 h compared to the control with no lactate or lysine ($P < 0.001$). However, inclusion of lysine and lactate in combination had higher growth of both subspecies (1-2 log increase) when compared to lysine or lactate alone ($P < 0.001$). Treatment and sampling time had a significant interaction ($P < 0.001$) and the inoculum concentration had no effect ($P = 0.08$). Our study demonstrated that lysine and lactate together were better than lysine or lactate alone in supporting the growth of *F. necrophorum*. The in vitro fermentation system will be valuable in evaluating antifusobacterial activities of novel antibiotic alternatives.

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Presenter: Harith Salih

Antibacterial activities of grape seed, green tea and rosemary phenolic extracts against pathogens that cause liver abscesses in feedlot cattle

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Keywords: Liver abscess, Feedlot cattle, Plant based phenolic extracts, Antibiotic Alternatives

Abstract

Liver abscesses occur in finishing cattle fed high-grain, low-roughage diets. Liver abscesses are of significant economic concern to the feedlot industry. The causative agents include *Fusobacterium necrophorum* subsp. *necrophorum, F. necrophorum* subsp. *funduliforme*, *Trueperella pyogenes*, and *Salmonella enterica*. Tylosin, a macrolide antibiotic, is supplemented in the feed to reduce liver abscesses. Because of the concern with emergence of potential antimicrobial resistance, there is a need to find antibiotic alternatives. Plant based phenolic compounds, which have antimicrobial activity, have the potential to be an antibiotic alternative to control liver abscesses.

We investigated the inhibitory activities of phenolic compounds extracted from grape seed, green tea, and rosemary on liver abscess-causing bacterial pathogens (5 strains each). Phenolic compounds were extracted by using 75% aqueous acetone as a solvent, and total phenolic content was determined spectrophotometrically. Anaerobic brain heart infusion broth (for Fusobacterium) and Muller-Hinton broth (for *S. enterica* and *T. pyogenes*) with phenolic extracts at 0, 0.1, 1, and 2 mg/ml were used. The treatment groups were in a 2-way factorial arrangement (bacterial strain and a phenolic extract). Growth was measured at 0, 12, 24 and 48 hours by determining bacterial concentrations [absorbance and log10(CFU/ml)]. A micro-broth dilution method was used to quantify the inhibition, if the compound was inhibitory.

Statistical analyses were performed using a PROC MIXED procedure with the DDFM=KR option in the MODEL statement (SAS version 9.4; Cary, NC). The least-squares means (LSMs) and their standard errors (SEMs) were used to assess the effects of phenolic extracts. All tests were two-sided and were conducted at the 0.05 level. Grape seed and green tea phenolics inhibited growth of both *Fusobacterium subspecies*, *T. pyogenes* and *S.*

enterica. Green tea at 1 mg/ml concentration was more effective in inhibiting the growth of *Fusobacterium* when compared to grape seed and rosemary ($P < 0.001$). Green tea at 2 mg/ml concentration showed greater inhibition of *Salmonella* ($P = 0.0089$) compared to 1 mg/ml. The inhibitory effect was dose dependent. The inhibitory effect was consistent across all strains within the same bacterial species. On quantification, the phenolic extracts were inhibitory against T. pyogenes with minimum inhibitory concentration ranging from 6.25-12.5 µg/ml. Further studies are ongoing to investigate different concentrations and stability of these plant based phenolic compounds at various temperature conditions on the liver abscess pathogens. Plant based phenolic compounds that inhibit the pathogens may have the potential to be supplemented in the feed to control liver abscesses.

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Presenter: Mina Abbasi

Liver abscesses in feedlot cattle: Is *Fusobacterium necrophorum* subsp. *necrophorum* concentrations in ruminal and colonic epithelial tissues related to occurrence of liver abscesses?

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Keywords: *Fusobacterium necrophorum*; liver abscesses; rumenitis; ruminal acidosis; *Salmonella*

Abstract

Liver abscesses (LA) pose a significant challenge in the feedlot industry, impacting cattle performance and carcass yield and quality. The onset of LA is associated with feeding of high-grain diets, which leads to chronic acidosis and subsequently to ruminitis. *Fusobacterium necrophorum* subsp. *necrophorum*, a ruminal bacterium, is the primary causative agent. Acid-damaged ruminal and possibly, colonic epithelium become susceptible to invasion and colonization by subsp. *necrophorum*. The colonization possibly leads to a continuous flow of bacterial emboli into the portal blood circulation. The liver acts as a filter and becomes a focal point for bacterial congregation, resulting in abscess formation. We hypothesize that the occurrence of LA is related to the presence and concentrations of subsp. *necrophorum* in ruminal and possibly colonic epithelial tissues. The primary objective of the study was to detect and quantify *F. necrophorum* in ruminal and colonic tissues in calves experimentally induced with LA using a nutritional model, which included intrarumianal inoculation of LA pathogens.

Calves (n=120) from three independent studies with five treatment groups, which included: 1. Basal diet, 2. Basal diet with intraruminal inoculation of subsp. *necrophorum*, 3. Acidotic diet, 4. Acidotic diet with intraruminal inoculation of subsp. *necrophorum*, and 5. Acidotic diet with intraruminal inoculation of subsp. *necrophorum*, and *S. enterica* serotype Lubbock. Calves were euthanized 3 weeks after the intraruminal bacterial inoculation. None of the calves fed basal or acidotic diet and no intraruminal inoculation of subsp. *necrophorum* developed LA. Calves fed basal or acidotic diet and inoculated intraruminally with subsp. *necrophorum* and or S. Lubbock developed LA (45/98). Samples collected at necropsy included abscesses (n=45), healthy liver (n=73), ruminal epithelial (n=118) and colonic (n=118) tissues. Samples were homogenized and subjected to culture method for isolation and real time (RT) PCR-based detection that targeted promoter region of the leukotoxin gene (lkt) for detection and quantification of subsp. *necrophorum*. The average concentration of subsp. *necrophorum* in

purulent materials of LA were 4.4×10^7. The concentrations of subsp. *necrophorum* in ruminal and colonic tissues of calves with LA were similar to that of calves with healthy livers. Our study revealed that intraruminal inoculation of subsp. *necrophorum* is required to induce LA and the occurrence of LA does not appear to be related to concentration of subsp. *necrophorum* in the ruminal or colonic tissues.

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Presenter: Mikaela Weeder

Comparison of firocoxib, meloxicam and transdermal flunixin for pain mitigation in meat goats with experimentally induced lameness

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Keywords: firocoxib, flunixin, goat, lameness, meloxicam

Abstract

Introduction: Lameness is a longstanding animal welfare issue in livestock production. A growing meat goat population increases the need for better analgesic options for lameness treatment in meat goats. Lack of NSAID knowledge in meat goats inhibits veterinarians from effectively treating lameness issues. This study sought to better understand NSAID effectiveness in meat goats with experimentally induced lameness.

Methods: Firocoxib (administered orally) (0.5mg/kg, 1.0mg/kg, 2.0mg/kg), meloxicam (administered orally) (1.0mg/kg, 2.0mg/kg, 3.0mg/kg), and topical flunixin (3.3mg/kg, 4.2mg/kg, 5.0mg/kg) were administered to individuals after lameness was induced (9 treatments total). A positive (lameness induction, no NSAID) and negative control (saline induction, no NSAID) were also implemented for comparison purposes. Treatment was administered at 0h. Lameness was induced in the left hind foot to begin. A minimum 2-week washout period occurred afterwards for each individual on trial. Lameness was induced in the right hind foot after the washout period was complete. Each individual received the same treatment for left and right hind foot induction. Outcomes measures for left and right hind feet included VLS (Visual Lameness Scoring), VAS (Visual Analog Scoring), IRT (Infrared Thermography), MNT (Mechanical Nociception Threshold), and blood plasma cortisol concentrations. Timepoints included -24, 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, and 72h.

Results: Negative control (non-lame) goats had lower VAS measures (23.5±5.2 mm) compared to positive controls (50.6±4.6mm) and goats receiving high dose firocoxib (48.3±4.9 mm; P = 0.005). Negative controls had lower temperature differences by IRT between the lame and sound leg (P = 0.003) compared to all other treatment groups. There was a significant treatment effect for MNT differences between the lame and sound limb with negative controls having lower differences. There were no observed treatment effects for cortisol levels between treatment groups (P = 0.57).

Conclusion: Overall, meloxicam at 2.0 mg/kg proved the most effective at treating short-term lameness in meat goats during this study. While some drug/dosage treatments provided some analgesic effect, meloxicam at 2.0mg/kg provided the most consistent analgesic effect for every timepoint after lameness induction. Further investigation is needed to better understand NSAID usage to treat lameness issues in meat goats.

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Presenter: Rebecca Bigelow, MS

Evaluating the efficacy of Maternal Bovine Appeasing Substance (MBAS) (FerAppease®) administration on performance outcomes in bottle-fed beef-on-dairy calves during weaning

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Keywords: weaning, calves, pheromone, maternal bovine appeasing substance

Abstract

Weaning is an unavoidable stressor for dairy and beef calves. Maternal bovine appeasing substance (MBAS) is a naturally secreted pheromone that induces an appeasing effect on nursing offspring. To reproduce MBAS' effect, a synthetic MBAS analog has been developed to decrease distress. Our objective was to determine if a single topical dose of MBAS would improve performance outcomes of beef-dairy calves during weaning process.

Twenty-six male beef-dairy calves were blocked by weight (72±6 kg) and randomized to 1 of 2 treatments: saline (CONT) or MBAS (MBAS). Calves received a single topical dose of the designated treatment (10 mL) on day -3 prior to step-down weaning process at 8 weeks of age. Calves were housed individually in raised crates in a climate-controlled room. Calf starter was offered and refusals were weighed and recorded twice daily. Bodyweights were collected every other day for 10 days following weaning. Average daily gain (ADG) and feed to gain conversions were calculated. Behavior, vocalization, and blood cortisol concentrations were assessed. A linear mixed model with fixed effects of time, treatment, and treatment × time was used to analyze performance variables. Calf was used as a random effect to account for repeated measures over time. Analysis additional outcomes are in progress.

When analyzing bodyweight and ADG, there was no treatment effect nor a time × treatment interaction between CONT and MBAS. Timepoint had a significant effect (P<0.01) on bodyweight and ADG.

While MBAS did not have an effect on calf performance, benefits of animal welfare will be assessed upon further analysis of remainder outcomes.

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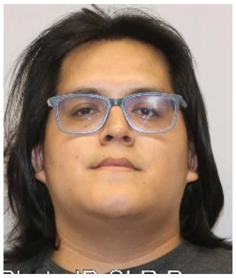

Presenter: Cesar Aparicio

Microwave-responsive thermosensitive lipid nanoparticles for spatiotemporal delivery of chemotherapeutics

Corresponding Author: Cesar Aparicio

Keywords: Hyperthermia; Ionic liquid; cancer

Abstract

Pancreatic cancer is a highly lethal malignancy with an urge for the exploration of novel treatments. One promising approach is microwaved-induced hyperthermia, which has shown great antitumor and synergistic effects when combined with other therapies. The application of microwaves produces heat by dielectric hysteresis which depends on the dielectric properties of molecules. Ionic liquids (ILs) are compounds composed of ions with <100 °C melting point and have strong dielectric properties. In short, we propose the creation of microwave-sensitive nanoparticles using IL, enabling precise hyperthermia induction with low-powered microwaves in localized areas. To achieve this, we stabilize a core composed of 1-butyl-methylimidazolium bromide or 1-butyl-3-methylimidazolium hexafluorophosphate using Span 80, Tween 20, and Triton-X as surfactants, along with phosphatidylcholine lipids as “cosurfactants”. Employing an emulsification procedure for fabrication, nanoparticles are characterized using dynamic light scattering, Fourier-transform infrared spectroscopy, and transmission electron microscopy. Furthermore, we assess microwave sensitivity by comparing thermal changes between liposomes loaded with 120 mM and 250 mM NaCl solutions. Subsequently, we evaluate biocompatibility and heat effects by incubating IL nanoparticles with BXPC3 and KPC cells, monitoring cell viability through MTT assays. We aim to confine the ILs within the nanoparticle core rather than incorporating them into the membrane. We anticipate increased microwave sensitivity, translating to reduced off-target effects and enhanced precision in hyperthermia treatment. This research holds promise for improving the therapeutic outcomes of pancreatic cancer treatment.

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Presenter: Erin Mayhue

Interaction of emodepside with canine MDR1: in silico and cellular assay

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Keywords: emodepside; canine; MDR1; resistance; hookworm

Abstract

Introduction: Emodepside is an FDA approved anthelmintic indicated for the treatment of gastrointestinal nematodes in cats. The feline product (Profender) has been used extralabel in dogs with resistant hookworm infection. There have been two reports of neurotoxicity in multidrug resistant 1 (MDR1) mutant dogs after receiving emodepside. There is a gap in our understanding if and how emodepside interacts with canine MDR1. We hypothesized that emodepside is transported by MDR1 as a substrate.

Methods: We evaluated this hypothesis by using three types of assays: in silico, toxicological, and functional. For in silico evaluation, we used ModWeb, PathDock, and PLIP to determine the theoretical binding sites of emodepside on modeled canine MDR1 protein. For both cytotoxicity and functional assays, two Madin-Darby canine kidney (MDCK) cell lines, one with MDR1 (wildtype, WT), one without MDR1 (knockout, KO), were grown to 95-100% confluency in 96 well plates. For the cytotoxicity assay, serial dilutions of emodepside were added to the cells and incubated at 37C with 5% CO_2 for 1 week. Crystal violet was then added and absorbance was recorded using a plate reader (A570nm). For the functional assay, emodepside dilutions and a fluorescent dye (H33342) were added and incubated for 2 hours. Fluorescence was then recorded using a plate reader (Ex/Em 361nm/486nm).

Results: Molecular modeling results showed that emodepside had multiple binding sites on the MDR1 protein. Emodepside (10uM) did not cause cell death in WT and KO cells and no differences were observed in toxic effects between the two cell lines. The IC50 of emodepside in the H33342 assay was ~2.8nM.

Conclusion: These results indicate that emodepside is a substrate of canine MDR1. In MDR1 mutant dogs given extra label emodepside for resistant Ancylostoma caninum, emodepside can cross the blood brain barrier. These dogs may be at risk of developing neurological side effects. Therefore, extra label use of the feline formulation of emodepside should be used with extreme caution in dogs.

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Presenter: Hanna Rhoads

Got Lyme?: Prevalence of *Borrelia burgdorferi* in Ixodes scapularis ticks collected from horses across the United States

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Keywords: Ixodes scapularis; Borrelia burgdorferi; Black-legged tick; National Equine Tick Survey

Abstract

Background: *Borrelia burgdorferi* is a spirochete transmitted by Ixodes spp. ticks and is responsible for Lyme in humans, dogs, and horses. Although I. scapularis ticks are present across most of the Eastern and Midwestern United States, the prevalence of *B. burgdorferi* in its tick vector is variable throughout the range, and there are few studies on the prevalence of this pathogen in ticks collected from horses.

Methods: As part of the National Equine Tick Survey (NETS), ticks were collected from horses across the country and morphologically identified as Ixodes scapularis. Submissions were sorted based on state of origin and previously established regions defined in other parasite epidemiologic studies. Ticks identified as I. scapularis were dissected, had their DNA extracted, and were tested via nested PCR for B. burgdorferi.

Results: A total of 328 adult male and female I. scapularis ticks were submitted; 92 from the Midwest (MW), 198 from the Northeast (NE), 38 from the Southeast (SE), and 0 from the West (W). Prevalence of B. burgdorferi was 20% overall, 23.2% for NE, and 15.8% for SE, and 15.2% for MW. The number of submissions was highly variable across states with 161 submissions from Pennsylvania and many enrolled states submitting zero or only one tick.

Conclusions: Higher number of submissions from states may reflect higher populations of I. scapularis as well as increased concern about tickborne disease. Further study is warranted to assess tick feeding behavior on horses and its relationship to *B. burgdorferi* prevalence.

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Presenter: Mahamudul Hasan

Online Survey on Canine Infectious Respiratory Disease Complex: Risk Factor, Tracking and Disease Pattern History from Dog Owners across the United States of America

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Keywords: Canine Infectious Respiratory Disease Complex (CIRDC); logistic regression analysis; survey

Abstract

Background: Canine Infectious Respiratory Disease Complex (CIRDC), commonly known as Kennel cough, is a highly contagious pathogen that affects the respiratory system of canine population. Traditionally, the primary causes responsible for CIRDC include Bordetella bronchiseptica, canine herpesvirus, canine adenovirus type 2, canine distemper virus, and canine parainfluenza virus. However, emerging pathogens such as canine pneumovirus, influenza virus, respiratory coronavirus, Streptococcus equi subspecies zooepidemicus, and Mycoplasma cynos are now associated with the onset of this disease complex. Recent findings indicate that certain organisms linked to CIRDC exhibit remarkable resilience, enduring vulnerable conditions for several weeks. Consequently, it is crucial to gain insights into the extensive epidemiological distribution of the disease across the country, its progression timeline, and the susceptibility of dogs.

Methods: The online cross-sectional study, conducted from November 24, 2023 to January 26, 2024, aimed to investigate the current cases of CIRDC across various states. Our survey targeted "2023 Canine Infectious Respiratory Disease Tracking" Facebook group members, and their responses were used for data collection. In total 482 complete responses were received and considered for inclusion in the final study. Data were systematically collected through a Google Form, and the responses were exported to Microsoft Excel for analysis. The questionnaire encompassed crucial aspects, including the age and breed of the dogs, the location and number of reported cases, the history of exposure, prior occurrences, and vaccination records. Additionally, the questionnaire also asked about the time interval between exposure and the onset of symptoms, the medical history, the track of treatment, and the final prognosis of survival or death. Survey questions underwent analysis using descriptive statistics. Various variables, considering biological plausibility such as age, prior respiratory disease status, venue of exposure, type of medication and vaccination history, were subjected to examination for their association with the outcomes of death or survival. The study employed both univariate and multivariate analyses to ascertain the independent risk factors associated with CIRDC.

Results: Upon analyzing the results, the current study revealed CIRDC cases were widespread, with the highest incidence and responses documented in California, followed by Colorado Utah, Pennsylvania, Wisconsin, Florida, and Massachusetts. Exposure locations where dogs congregated, such as dog events, kennels/boarding facilities, and dog parks, were identified from most of the cases. In terms of symptom onset, most dogs exhibited signs within 10 days, with the highest number reporting symptoms five days post-exposure. In the context of univariate and multivariable logistic regression analysis, treatment and vaccinations status variables were identified as an independent factor for patient mortality.

Conclusion: This current study serves as an introductory exploration of a CIRDC, laying the groundwork for larger cross-sectional surveys, facilitating a comprehensive examination of the actual risk factors associated with a CIRDC. The insights gained from these subsequent studies can further enhance our understanding of the disease dynamics, treatment strategy and vaccination strategy contributing to the formulation of targeted interventions and preventive measures.

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Presenter: Mehrnaz Ardalan

Determination of seroprevalence for SARS-CoV-2 in farmed and wild white-tailed deer

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Keywords: SARS-COV-2, Deer, Antibody

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a Betacoronavirus of the family Coronaviridae. The emergence of new SARS-CoV-2 variants of concern (VOCs) has further expanded the broad host range which has been described for SARS-CoV-2, creating concern for the establishment of virus reservoirs in animals with additional genetic evolution resulting in decreased vaccine efficacy, increased transmission rates, and further global economic losses. Improved serological detection methods for antibodies against both the nucleocapsid (N) and spike (S) proteins of SARS-CoV-2 are crucial for estimating the prevalence of infection in humans and animals. White-tailed deer (WTD) are highly susceptible to SARS-CoV-2 and are prevalent throughout the United States with a high population density. Sustained transmission of SARS-CoV-2 in wild and farmed WTD poses a risk to humans in close contact with this species. To address this concern, in the present study, a total of 312 serum samples were collected between 2018 - 2022 from Ohio (n= 126; wild WTD), Indiana (n= 60; farmed WTD), and Kansas (n= 126; wild and farmed WTD) and analyzed for the presence of SARS-CoV-2-specific antibodies. The testing was performed using two commercial and one in-house ELISA assay based on: (i) the inhibition of the SARS-CoV-2 receptor-binding domain (RBD)– angiotensin-converting enzyme 2 (ACE2) interaction (cRBD-ELISA);

(ii) the nucleocapsid protein (cN-ELISA) using a commercial kit; and (iii) an indirect ELISA (RBD iELISA) based on the RBD protein, which was developed in-house. Conventional virus neutralization tests (VNTs) using the Wuhan-like SARS-CoV-2 USA/WA1/2020 isolate, the VOC Omicron BA.2, or the VOC Delta (B.1.617.2) were used as reference assays. Our results indicated that 7.9 % (10/126), 1.7% (1/60), and 0% [(0/16) of wild WTD] samples from Ohio, Indiana, and Kansas, respectively, were seropositive in the cRBD-ELISA, whereas 3.2% (4/126), 1.7% (1/60), and 6.3 % [(1/16) of wild WTD] samples from Ohio, Indiana, and Kansas, respectively, tested positive by the RBD iELISA. In addition, 4% (5/126), 5% (3/60), and 0% [(0/16) of wild WTD] samples from Ohio, Indiana, and Kansas, respectively, were positive by the cN-ELISA. From the 110 tested farmed WTD samples, 95% (105/110), 86% (95/110), and 53% (58/110) tested positive in the cRBD-ELISA, the RBD iELISA, and the cN-ELISA, respectively. In comparing the results obtained from iELISA with VNT, the diagnostic sensitivity and specificity of the in-house RBD ELISA were determined to be 97% and 90%, respectively. These results demonstrate a strong correlation between the RBD-based iELISA and the VNT, validating the use of the iELISA for the rapid detection of SARS-CoV-2-specific antibodies in WTD. Interestingly, the highest seropositivity among WTD was observed in farmed animals in the state of Kansas. Further research is necessary to determine seroprevalence of SARS-CoV-2 in WTD across the United States.

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Presenter: Mehrnaz Ardalan

Determination of SARS-CoV-2 seroprevalence in cattle, sheep, and goats using different ELISA tests

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Keywords: SARS-Cov-2, Ruminants, Antibody

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded, enveloped RNA virus belonging to the Coronaviridae family, genus Betacoronavirus. After emerging in late 2019, SARS-CoV-2 spread rapidly through human-to-human transmission, resulting in the global COVID-19 pandemic. In the past 3 years, a multitude of domestic and wild animal species have been identified to be susceptible to SARS-Cov-2 infection through experimental inoculation and/or animal surveillance activities. Therefore, reliable diagnostic assays are required to estimate the seroprevalence of SARS-Cov-2 in animal populations. Previous SARS-CoV-2 experimental challenge studies in cattle, sheep, and goats suggest that these ruminant species have a rather low susceptibility to infection. However, newly emerging SARS-CoV-2 variants of concern (VOCs) have demonstrated changes in host range, warranting continued surveillance of SARS-CoV-2 in animal populations. Detection of SARS-Cov-2 specific antibodies in animals is an important surveillance tool since viral shedding in animals can only be detected for a short period of time. In the present study, serum samples collected from cattle (n=691), sheep (n=698), and goats (n=707) from 2019 to 2022 from several geographically distinct regions in the United States were analyzed

for the presence of SARS-CoV-2-specific antibodies. The testing was performed using two commercial ELISA assays based on: (i) the inhibition of the SARS-CoV-2 receptor-binding domain (RBD)– angiotensin-converting enzyme 2 (ACE2) interaction (cRBD-ELISA) and (ii) the nucleocapsid protein (cN-ELISA) of SARS-CoV-2; the cRBD-ELISA detects surrogate neutralizing antibodies, whereas the cN-ELISA detects non-neutralizing antibodies against SARS-CoV-2. Positive results from the cRBD-ELISA were confirmed using a conventional virus neutralization test (cVNT) employing the Wuhan-like SARS-CoV-2 USA/WA1/2020 isolate as a reference assay. Our results indicate that ~1% (6/691) of cattle, ~2% (13/698) of sheep, and ~2.5% (18/707) of goat serum samples were positive in the cRBD-ELISA, whereas ~4% (25/691) of cattle and sheep (27/698), and 2.5% (18/707) of goat serum samples tested positive by the cN-ELISA. None of the cRBD-ELISA positive cattle, sheep, or goat serum samples had detectable neutralizing activity (> 1:8) against the SARS-CoV-2 USA/WA1/2020 isolate by the cVNT. Our results demonstrate low seropositivity in cattle, sheep, and goats in the U.S., indicating the importance of continues monitoring for SARS-CoV-2 prevalence in animal species that are in close contact with humans.

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Presenter: Sarah DeVader

Water extract from Euglena gracilis attenuates lung cancer growth in mice by increasing PD-1+ CD8+ effector tumor infiltrating lymphocytes

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Keywords: lung cancer; immune checkpoint proteins; PD-1; tumor infiltrating lymphocytes

Abstract

Introduction: Lung cancer is the leading cause of cancer related death in the US. In human lung cancers, high tumor infiltration of lymphocytes and high expression of the immune checkpoint proteins programmed death-ligand 1 (PD-L1) and programmed death 1 (PD-1) in early stages is linked to susceptibility to immune checkpoint inhibitor (ICI) therapy and shown to increase survival rate after ICI therapy. Our previous studies revealed daily oral administration of Euglena gracilis water extract (EWE) significantly attenuated lung cancer growth in mice. EWE also stimulates T cell-induced cytolysis of cancer cells in vitro.

Here, we investigated the relationship between EWE-induced attenuation of lung cancer growth and EWE-induced T cell stimulation with a special interest in PD-1 and PD-L1 expression in a murine lung cancer model.

Methods: Mouse Lewis Lung Carcinoma (LLC) cells were treated with EWE or PBS in vitro and the mRNA was collected for real-time PCR . The LLC cells were also stained for flow cytometry to analyze the expression of both PD-1 and PD-L1. In addition, C57BL/6 mice were treated daily with EWE or PBS through drinking water for three weeks, then LLC cells were injected via tail vein to induce tumors in the lung. After another three weeks of treatment, the mice were sacrificed, and the lung tumors were collected. These lung tumors were processed by immunohistochemical or immunofluorescent staining with antibodies for PD-1, PD-L1, and cytotoxic T cell marker CD8.

Results: This study found that treatment with EWE in vitro increased both mRNA and surface protein expression of PD-1 and PD-L1 in murine lung carcinoma cells. In the mouse study, EWE was revealed to increase the amount of PD-1 and PD-L1 mRNA and proteins in murine tumor tissues, which was correlated with a decrease in tumor weights. This study also discovered that EWE primarily increases the PD-1 expression in tumor infiltrating lymphocytes.

Conclusion: These results suggest there is a correlation between the EWE-induced attenuation of lung cancer growth and the EWE-induced increase of PD-1 expression in tumor infiltrating lymphocytes. However, further research is needed to clarify the role of these tumor infiltrating lymphocytes with increased PD-1 expression and the effects of increased PD-1 and PD-L1 expression seen in the LLC cells themselves. Further research is also recommended to determine the mechanisms by which EWE stimulates PD-1 and PD-L1 expression in both tumor cells and lymphocytes, and the potential influence EWE has in the efficacy of ICI therapy against lung cancer.

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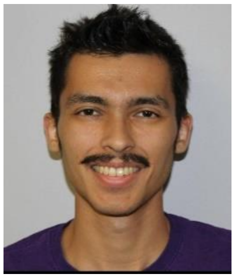

Presenter: Brian Wolfe

Biological sex differences in disease severity, lethal doses, and antibody responses after infection with H1N1 and H3N2 influenza A viruses in a mouse model

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Keywords: Influenza A virus, sex differences, sex-specific pathophysiology

Abstract

Introduction: Biological sex, i.e., being male or female as determined by sex chromosome complements, gonadal hormones, and their interactions, impacts influenza virus pathogenesis and immunity to vaccines. In this study, we aimed to establish a mouse model to better understand the effects of biological sex during influenza A virus (IAV) pathogenesis. Our objectives were to determine the lethal doses, compare morbidity and virus titers, and evaluate antibody responses between male and female mice after infection with H1N1 and H3N2 IAVs.

Methodology: Adult (8-10 weeks old), male and female C57BL/6 mice purchased from the Jackson laboratory were inoculated with 101 to 105 tissue-culture infectious dose (TCID50) of mouse-adapted A/California/04/09 H1N1 (refereed as maH1N1) or A/Hong Kong/1/68 H3N2 (referred as maH3N2) IAVs. The virus was diluted in 30 µL inoculum and administered through the intranasal route under ketamine and xylazine anesthesia. Body mass and temperature were recorded every day until 14 days post inoculation (dpi) or the humane endpoint of 25% body mass loss was reached for each mouse. Mouse lethal dose (mLD50) was calculated for males and females against both viruses using a dose-response curve and nonlinear regression model. Morbidity data were compared using two-way repeated measures ANOVA followed by Tukey's multiple comparisons. Survival data were compared using Kaplan-Meier's simple survival analysis. Data were analyzed in GraphPad Prism 10.1.0 and p<0.05 was considered a significant difference.

Results: After infection with maH1N1 IAV, all male mice survived and recovered up to a dose of 102 TCID50 while females showed survival and recovery only up to 101.5 TCID50. Likewise, after infection with the maH3N2 virus, all infected males survived and recovered up to a dose of 103 TCID50 while females showed survival and recovery only up to 102 TCID50. Subsequently, mLD50 for both viruses was lower for females compared with the male mice. The mLD50 for maH1N1 virus was 101.53 TCID50 for females and 102.50 TCID50 for males. Likewise, mLD50 for maH3N2 virus was 102.45 TCID50 for females and 103.57 TCID50 for males. When infected with 101.5 TCID50

of maH1N1 and 102 TCID50 of maH3N2 viruses (i.e., the doses in which all mice survived in both males and females), female mice lost significantly greater body mass compared to males. Experiments are ongoing to determine the differences in pulmonary pathology, cytokine responses, and lung virus titers in mice euthanized at 3 dpi and plasma antibody responses at 14 dpi in recovered mice.

Conclusion: We have established a mouse model at KSU to study the biological sex differences during IAV infection. Adult females suffer greater morbidity and mortality after infection with different subtypes of IAVs. As biological sex impacts morbidity, mortality, and antibody responses to IAVs, biological sex and underlying mechanisms for the differential outcomes should be considered during the design of influenza vaccines and therapeutics.

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Presenter: Saurav Pantha

Establishment of a mouse model of diet-induced obesity to study the effects of interaction of biological sex and obesity during influenza A virus pathogenesis

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Keywords: Diet-induced obesity, influenza A virus, sex difference

Abstract

Introduction: In this study, our objectives were to establish a mouse model of diet-induced obesity (DIO) for both sexes and to evaluate the interaction of biological sex and obesity during IAV infection in DIO mouse model.

Methodology: Male and female C57BL/6 mice (n=20/sex, 5-weeks old) obtained from the Jackson laboratory were fed with either a high-fat diet (60% fat diet) or a control diet (10% fat diet) between the 6th to 14th weeks. Body mass was measured every week and mouse that gained more than 20% body mass compared to age and sex-matched mice on control diet was defined to be obese. Glucose tolerance test (GTT) was carried out at 14th week after putting mice on 6 hours fasting followed by intraperitoneal injection of 25% glucose solution and subsequent measurement of blood glucose level at 0, 15, 30, 60 and 120 minutes after glucose administration. On the 15th week, mice were inoculated either with Dulbecco's Modified Eagle Medium (DMEM) or 103 tissue-culture infectious dose 50 (TCID50) of mouse-adapted H1N1 IAV through intranasal route. Mice were monitored for changes in body weight and rectal temperature for 3 days post inoculation (dpi) and then euthanized at 3 dpi to collect lungs and other tissues for virus titration and histopathology analysis. During euthanasia, weight of inguinal adipose tissue (IAT), gonadal adipose tissue (GAT), and perirenal adipose tissue (PAT) were also measured.

Results: After 14 weeks, 100% of the male mice on high-fat diet responded to the diet treatment and became obese while only 70% of the female mice on high-fat diet responded to the diet treatment. There were 30% non-responder (non-obese) females and despite being on high-fat diet they had weekly body mass gain, blood glucose levels, and body fat measurements comparable to female mice on control diet. While male mice on high-fat diet gained significantly greater body mass within 2nd week of diet treatment, responder females on high-fat diet took

6-weeks of diet treatment for that. At 14 weeks, both males and responder females on high-fat diet treatment had significantly greater blood glucose levels. For males, high blood glucose persisted at 30, 60, and 120 min while for females it was evident at 15, 30, and 60 min after glucose treatment. At 15th week, males as well as responder females on high-fat diets had significantly higher weights of IAT, GAT and PAT compared to male and female mice on control diet. After infection with influenza virus, there was no difference in change in body mass at 3dpi between male and females in control diet compared with the responders in high-fat diet. Experiments are ongoing to determine lung virus titers, pulmonary pathology, differences in cytokines and adipokines and sex steroid levels.

Conclusion: We have established a mouse model of diet-induced obesity at KSU. While male mice develop obesity efficiently, females gain weight slowly and have around 30% non-responders. Future studies will explore the interaction of biological sex and obesity during IAV infection and vaccination.