THE SOCIETY OF PHI ZETA

ALPHA IOTA CHAPTER

Third Annual

Phi-Zeta Research Day

February 21, 2025

SCHOOL OF
**VETERINARY
MEDICINE**

TEXAS TECH

Acknowledgments

On behalf of the Phi Zeta Research Day organizing committee, we extend our deepest gratitude to all those who contributed to making this event a success.

We sincerely thank our abstract **reviewers, judges, and moderators:**

> Drs. Howard Rodriguez Mori, Meredyth Jones, Blaine Johnson, Michael Cruz Penn, Marcelo Schmidt, Elpida Artemiou, Klementina Fon Tacer, Stephanie Myers, Heidi Villalba, Babafela Awosile, Carolyn Arnold, Annelise Nguyen, Kellie Littrell, Nanny Wenzlow, Prasanth Chelikani, Devendra Shah, Nichole Anderson, Soni Khandelwal, Olufemi Akinkuotu, Britt Conklin, John Gibbons, Fernanda Rosa, and Smriti Shringi.

We also extend our appreciation to the **volunteers who assisted with the event logistics:**

> Heather Scalf, Soni Khandelwal, Travis Sills, Henry Granda, Weston Brooks, Karen Michael.

Special thanks to our **generous sponsors** for their continued support for this event.

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Texas Tech University School of Veterinary Medicine

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Finally, we are grateful to all participants for sharing their research and engaging in meaningful discussions. Your enthusiasm and commitment to advancing veterinary and biomedical sciences are truly inspiring.

Thank you for being part of Phi Zeta Research Day.

2025 Phi Zeta Research Day Organizing Committee:

President (Smriti Shringi**) and Secretary/Treasurer (**Fernanda Rosa**)**

The Alpha Iota Chapter, The Society of Phi Zeta
The Honor Society of Veterinary Medicine
Alpha Iota Chapter, SVM TTU

From	To	Description			
8:00 AM	9:00 AM	Presenters and Judges Check in			
9:00 AM	10:45 AM	**Graduate Student - Oral Presentations** **Location: E106**	9:00 AM	9:13 AM	Samuel Ajulo
			9:13 AM	9:26 AM	Tara Bayat
			9:26 AM	9:39 AM	Luciana Kluppel
			9:39 AM	9:52 AM	Vishakha Kulkarni
			9:52 AM	10:05 AM	Denis Stepihar
			10:05 AM	10:18 AM	Sima Tozandehjani
			10:18 AM	10:31 AM	Tomas Lugo
			10:31 AM	10:44 AM	Yamima Tasnim
10:44 AM	11:00 AM	BREAK - Coffee/Snack			
11:00 AM	12:00 PM	**Graduate Student - Poster Presentations, Location: ASCO Hall**			
12:00 PM	12:30 PM	LUNCH			
12:30 PM	1:30 PM	**KEYNOTE Seminar** **Dr. Don Knowles**	**“A Mentored Road of Trial, Error and Occasional triumph in Biomedical Research”**		
1:30 PM	1:40 PM	BREAK			
1:40 PM	2:45 PM	**DVM Student - Oral Presentations** **Location: E106**	1:40:00 PM	1:53:00 PM	Kae Szafranski
			1:53:00 PM	2:06:00 PM	Alexandria Lane
			2:06:00 PM	2:19:00 PM	Hailey Moore
			2:19:00 PM	2:32:00 PM	John Wofford
			2:32:00 PM	2:45:00 PM	Hannah Sieben
2:45 PM	3:00 PM	BREAK			
3:00 PM	4:00 PM	**DVM Student - Poster Presentations, Location: ASCO Hall**			
4:00 PM	4:30 PM	BREAK - Coffee/Snack			
4:30 PM	5:00 PM	**Awards & Induction of new members**			

KEYNOTE SEMINAR: "*A Mentored Road of Trial, Error and Occasional Triumph in Biomedical Research*"

Dr. Knowles holds a B.S. (1978, 1980) and D.V.M. (1982) from the University of Illinois and a Ph.D. (1988) from Washington State University. He is a Diplomate of the American College of Veterinary Pathologists (1990). Throughout his career, he has received numerous honors, including induction into the USDA-ARS Scientific Hall of Fame, the American Association of Equine Practitioners' Research Award, Camptender Award, American Sheep Industry, and election as a Fellow of AAAS.

Dr. Don Knowles has had a distinguished career in veterinary infectious disease research. He is currently a Board Member of US Animal Vaccinology Research Coordination Network at USDA-NIFA, an Adjunct Professor in the Department of Veterinary Microbiology and Pathology at Washington State University and an Affiliate Professor in the Department of Comparative Medicine in School of Medicine at University of Washington. His expertise spans federal, state, and private sectors, beginning with the United States Department of Agriculture (USDA) in 1986 and then Washington State University (WSU) in 1988 and Veterinary Medical Research & Development (VMRD) in 2017. Dr. Knowles has served as the Research Leader of the Animal Disease Research Unit (ADRU), of ARS-USDA, Location Coordinator for USDA activities at Washington State University, Pullman and University of Idaho, Moscow Campuses, and Affiliate Professor within the Department of Comparative Medicine with the University of Washington Medical School. He served as Research Leader of the Animal Disease Research Unit for 22 years (1995-2017) and played a key role in advancing research on viral, bacterial, prion, and protozoan diseases affecting livestock. He has served as an OIE expert for small ruminant lentiviruses and equine piroplasmosis and an elected US representative to the Scientific Committee of the STAR-IDAZ International Research Consortium on Animal Health. He has held significant positions, including Senior Level Executive Scientific Staff (ST-USDA) in 2005, Professor at WSU in 2000, and Chief Scientific Officer at Veterinary Medical Research & Development (VMRD) in 2017.

Dr. Knowles has trained 18 DVM PhD students through a collaborative state-federal program and contributed extensively to veterinary education, teaching virology, pathology (neuropathology), and immunology in the WSU-DVM program from 1988 to 2016. His research has led to over 239 peer-reviewed publications and multiple patents, including advancements in contemporary diagnostic technologies and disease control strategies. Notably, his patent application on SARS-CoV-2 surveillance facilitated uninterrupted manufacturing operations during the COVID-19 pandemic.

His research on infectious disease pathogenesis has resulted in significant advancements in vaccine development for East Coast Fever and Malignant Catarrhal Fever. He collaborated with regulatory agencies, the biomedical industry, and livestock organizations, such as the National Cattlemen's Beef Association and the American Horse Council, to further strengthen but also make his contributions significantly relevant to animal health.

His ongoing goal is to work with collaborative teams in veterinary medicine toward improving veterinary medical education and animal health.

Oral Presentations (Graduate students) – morning session:

Title	Presenter	Time	Room
Antibiotic Classes Influence the Evolutionary Dynamics of Multi-drug Resistance Traits	Samuel Ajulo	9:00 AM	E106
mRNA Translation is Disrupted upon Loss of Magel2 in the Mouse Hypothalamus and Pituitary in the Neuroendocrine Disorder Prader-Willi Syndrome	Tara Bayat	9:13 AM	E106
Anti-aging protein and non-coding RNAs are present on human triple-negative breast cancer and canine mammary carcinoma and can be used as potential prognostic markers and therapeutic targets.	Luciana Kluppel	9:26 AM	E106
Development of digital PCR assay for the detection of Taylorella equigenitalis, causative agent of Contagious Equine Metritis in Equine	Vishakha Kulkarni	9:39 AM	E106
Investigating the role of Magel2 during nutritional stress using single nucleus RNA sequencing	Denis Stepihar	9:52 AM	E106
MAGEA3/6 Enable Pancreatic Cancer Cell Survival and Growth under Nutrient Stress	Sima Tozandehjani	10:05 AM	E106
Impact of Antineoplastic Drugs on Gap Junction-Mediated Cell Communication in Co-Cultured Colorectal Cancer Spheroids	Tomas Lugo	10:18 AM	E106
Comparative phylogenomics of beta-lactamase-producing Escherichia coli at one health interface.	Yamima Tasnim	10:31 AM	E106

Oral Presentations (DVM students) – afternoon session:

Title	Presenter	Time	Room
Comparison of three diagnostic tests for arrival immunity status on future outcomes of beef-dairy calves.	Kae Szafranski	1:40 PM	E106
Assessing Ultrasound Probe Disinfection in Veterinary Clinics: A Hidden Risk for Bacterial Transmission?	Alexandria Lane	1:53 PM	E106
Ischemic Stroke Recovery of a Type 2 Diabetic Preclinical Female Model	Hailey Moore	2:06 PM	E106
Comparison of Simple Continuous Surgical Pattern versus Vest-Over-Pants Surgical Pattern for Linea Alba Closure in Cattle	John Wofford	2:19 PM	E106
Evaluating the impact of scratchers on fearfulness and claw health for cats in confinement	Hannah Sieben	2:32 PM	E106

POSTER Presentations (Graduate students) – morning session:

All presenters must be at their poster at ASCO HALL from 11:00 AM until 12:00 PM.

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Maternal Pea Fiber Supplementation Improves Energy Balance, Glycemic control and Cardiac Function in Spontaneously Hypertensive Stroke-Prone Rats and Their Offspring	Md Tareq Aziz	#1
Extracellular vesicles isolation and characterization approaches from bovine colostrum and its impacts in neonatal dairy calves' immune system	Rafaela Rodrigues Dos Santos	#2
Foodborne Pathogen Management: Targeting different Salmonella serovars with Acid-Producing Bacteria Combinations	Maria Duarte	#3
Public Perceptions of Calf Disbudding Techniques Used on Texas Farms	Andrea Calix	#4
Fecal microbiome of horses under different housing environments in West Texas	Md Kaisar Rahman	#5
The Quantification of Myo-Inositol in Swine	Emerald Julianna Salinas	#6
Authenticity of Cinnamon Verum Determined by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) and Machine Learning	Sumon Sarkar	#7
Melanoma antigen genes in testicular physiology and cancer: Protective roles on male germ cells during stress and immune response	Farzana Popy	#8
Overcoming Language Barriers in Animal Welfare: Spanish for Specific Purposes Courses and Internship Practice with Veterinary and Animal Science Students in Spanish-Speaking Farm Settings	Allen Jimena Martinez Aguiriano	#9
Meta-Analysis of Biofilm Production and Its Potential Link in the Successful Persistence of Non-typhoidal Salmonella in Food Chain	Asmita Shrestha	#10
Development of Species Identification Keys of Hair Morphology of Mammals of Taiwan Using the Light Microscope and Scanning Electron Microscope	Ching-Yun Hsu	#11
Anatomical angles of the rider influences Equine-Assisted Service horse gait symmetry	Bethany Baxley	#12
Decoding Diet: Uncovering Links Between Canine Nutrition and Gastrointestinal Health	Christopher Childress	#13
Targeted Communication & Educational Training for Farm Animal Veterinarians and Students: Education to Enhance Feedlot/Dairy Adherence to Postmortem Procedures	Nathaly Vargas Arroyo	#14

POSTER Presentations (DVM students) – afternoon session:

All presenters must be at their poster at ASCO HALL from 3:00 PM until 4:00 PM.

Title	Presenter	Poster Board
The use of a fixed visual reference point for determining veterinary students' ability to identify forelimb lameness.	Ally Vaughn	#15
Establishing a Renewable Frozen Tissue Biobank: Enhancing Research Longevity and Sustainability	Micah Rainey	#16
Genotypic and phenotypic characterization of fecal beta-lactamase-producing Citrobacter sedlakii isolated from horses	Charlotte Stanley	#17
Measurement of urinary electrolytes in healthy goats and those with urolithiasis	Kailey Clarke	#18
Effects of Myo-Inositol on Energy Balance in Female Rats	Zachary Eason	#19
qPCR evaluation of the fecal microbiome in healthy horses and those with colic	Makayla Richardson	#20
Flipped Classroom vs. In-Person Lab Demo to Improve Veterinary Students Short-Term Retention	Hailee Conley	#21
Impact of colostrum deprivation on ileal epithelia markers and inflammatory genes in beef-on-dairy calves	Kagan Migl	#22
Postmortem onset and progression of rigor mortis and changes in body temperature in calves and dogs	Paige Jones	#23
The use of transcutaneous ultrasound to assess the gastrointestinal motility at 3 sites of the ascending colon.	Aleksander Burr	#24
The use of a fixed visual reference point in determining veterinary students' ability to identify hindlimb lameness in horses.	Hunter Metzger	#25
Hematological parameters in beef-on-dairy calves induced to systemic inflammation during the neonatal period	Keerra Holzapfel	#26
Equine melanoma: Development of in vitro models for therapeutic and mechanistic studies	Emily Kibodeaux	#27
Evaluating melanoma antigens (MAGE) in equine melanoma compared to healthy tissues	Kayla Justiss	#28
Validation of a multi-purpose surgical model: "Popeye the Pug"	Erica Vincent	#29
Effects of Coastal Bermudagrass; fresh or hay, on equine fecal particle size, moisture content, and fiber content	Elise Marshall	#30
Vaccine Efficacy of Non-Vaccinated Beef Cattle Fed Mycotoxin Blighted Feed	Mackenze Brister	#31

2025 Phi-Zeta Abstracts

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Antibiotic Classes Influence the Evolutionary Dynamics of Multi-drug Resistance Traits

Samuel Ajulo[1], Smriti Shringi[1], and Devendrah Shah[1]

School of Veterinary Medicine, Texas Tech University, Amarillo, Texas

OBJECTIVE: Antibiotic resistance is a major 21st century challenge. Studies using single antibiotics in resistant bacteria suggest that antibiotic use selects resistant bacteria and may co- select for resistance to other antibiotics. However, these findings often lack real-world insights. This study investigates how antibiotic classes influence AMR evolution in multidrug-resistant pathogens using laboratory evolution models and National Antimicrobial Resistance Monitoring System (NARMS) epidemiological data.

METHODS: Duplicate clonal lineages of multidrug-resistant *Salmonella* Kentucky MDR-SK198 were passaged for 4000+ generations with or without five antibiotics (amoxicillin, chloramphenicol, ciprofloxacin, sulfamethoxazole-trimethoprim, and tetracycline) in intestinal-mimicking media. AMR phenotype (disk diffusion and minimum inhibitory concentration/MIC testing) and AMR genotype (Whole genome sequencing and RESFinder) changes were analyzed in evolved lineages. Epidemiological data from 10,861 human non-typhoidal *Salmonella* strains (1994–2024, National Antimicrobial Resistance Monitoring System (NARMS)) were analyzed using multivariate logistic regression to model co-resistance among antibiotic classes.

RESULTS: AMR phenotypes and genotypes of evolved lineages demonstrate an antibiotic-dependent evolution. Ciprofloxacin and streptomycin accelerated the loss of non-target AMR traits, while sulfamethoxazole-trimethoprim, amoxicillin, and tetracycline maintained their non-target AMR traits. No-antibiotic passage showed varied persistence. The Epidemiological investigation confirmed that quinolone resistance is associated with reduced resistance to other antibiotic classes, and a reverse case was observed for sulfonamide resistance ($p < 0.05$).

CONCLUSIONS: Our investigation provides insight into how antibiotic class/type influences AMR evolution and dynamics. This knowledge can guide us to choose antibiotics with a reduced potential for co-resistance, thus helping curb the spread of multidrug resistance.

mRNA Translation is Disrupted upon Loss of Magel2 in the Mouse Hypothalamus and Pituitary in the Neuroendocrine Disorder Prader-Willi Syndrome

Tara Bayat,[1,2] Maria Camila Hoyos Sanchez[1,2], Cristian Camilo Rodriguez Almonacid[3,4], Elena B Tikhonova[3], Juan Solano[1,2], Farzana Popy[1,2], Stephanie Myers[1], Andrey L. Karamyshev[3], Zemfira N. Karamysheva[3], and Klementina Fon Tacer[1,2]

Institution(s): [1] School of Veterinary Medicine, Texas Tech University, 7671 Evans Dr., Amarillo, TX

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Abstract:

Objective: Control of mRNA translation on the ribosomes provides a critical layer in gene expression regulation and is important in secretory cells, particularly in the hypothalamus. Dysregulation of mRNA translation contributes to several neurodevelopmental diseases. However, its contribution to the pathogenesis of Prader-Willi syndrome (PWS) is not known. PWS is a neurodevelopmental disorder, whose hallmark is hypothalamic neuroendocrine dysfunction. PWS is caused by loss of several genes, including melanoma antigen L2 (*MAGEL2*), which is critical for the regulated secretion of hypothalamus. We hypothesize that MAGEL2 contributes to proper hypothalamic neuroendocrine function also on the translational level. **Methods:** To investigate the potential role of MAGEL2 in regulation of translation, we performed polysome profiling on the hypothalamus and pituitary of 16 wild-type and *Magel2*$^{p\Delta/m+}$ mice to separate mRNAs associated with different numbers of ribosomes, including monosomes, light polysomes, and heavy polysomes, which correspond to translation efficiency from low to high. We extracted RNA from each group, performed RNA sequencing and bioinformatic analysis. **Results:** Data analysis showed the differentially translated transcripts in KO vs. normal tissues. We observed differential translation of several mRNAs in the *Magel2*$^{p\Delta/m+}$ hypothalamus and pituitary, including 746 and 2293 transcripts in the pituitary and hypothalamus light polysomes, respectively. Notably, several hormones, including Prolactin and Oxytocin were differentially translated, suggesting that Magel2 contributes to the regulation of translation in these tissues. **Conclusions**: Our data provide novel insight into the role of MAGEL2 in protein synthesis and hormone secretion with implications for therapeutic interventions in patients with PWS and similar syndromes.

Acknowledgements/Funding:

This work was supported by the Foundation for Prader–Willi Syndrome Research Grant 22-0321 and 23-0447 (to K.F.T. and Z.K.), Texas Tech University start-up (to K.F.T.), and Cancer Prevention and Research Institute of Texas Scholar Award RR200059 (to K.F.T.).

Anti-aging protein and non-coding RNAs are present on human triple-negative breast cancer and canine mammary carcinoma and can be used as potential prognostic markers and therapeutic targets.

Luciana Kluppel[1], Tanja Plavec[2,3], Tanja Švara[2,3], Vladimira Erjavec[3], Joseph Emmons[4], Heather Tilman[4], Alicia K. Olivier[5], Fernanda Rosa[1] and Klementina Fon Tacer[1]

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[4] Veterinary Pathology Core, St. Jude Children's Research Hospital, Memphis, TN, USA
[5] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Starkville, MS, USA

Background: Human Breast Cancer (HBC) and Canine Mammary Carcinoma (CMC) share similarities such as cellular and molecular markers, and genetic alterations. Both conditions exhibit similar prevalence rates, with breast cancer being the most common cancer in women and mammary carcinoma representing the biggest concern in cancer for non-spayed female dogs. The high mortality rates of CMC, coupled with the limited treatment options for aggressive triple-negative breast cancer (TNBC), underscore the urgent need for early diagnostic markers and novel therapeutic targets. Klotho proteins, involved in aging and metabolism, have been implicated in cancer development. αKlotho and βKlotho function as tumor suppressors, while γKlotho appears to act as oncogene. However, the role of γKlotho in cancer remains poorly understood. **Objective:** Investigate the expression of γKlotho in both TNBC and CMC and explore its correlation with miRNA signatures to better understand its function and regulation in both species.

Methods: We isolated RNA and miRNA from tumors and benign mammary tissues of female dogs (n=31), and CMC cell lines (CF41.Mg; CMT-U27). The mRNA expression profiles were quantified using RT-qPCR, RNA sequencing, and microRNA sequencing. **Results: γKlotho** expression of CMC in normal mammary gland was higher compared to normal tissue. MicroRNA sequencing revealed four **miRNAs** were overexpressed, and eleven downregulated in tumor tissues. **Conclusions:** We have found γKlotho and a set of miRNAs as a potential promising novel diagnostic and stratifying markers for diseases prognosis in CMC with implication for human TNBC.

Development of digital PCR assay for the detection of *Taylorella equigenitalis*, causative agent of Contagious Equine Metritis in Equine

Vishakha Kulkarni [1], Smriti Shringi [1], Devendra H. Shah [1]

Affiliations: School of Veterinary Medicine, Texas Tech University, Amarillo, TX 79106

OBJECTIVE:
Contagious Equine arthritis (CEM), caused by Taylorella equigenitalis (TE), is a highly contagious, sexually transmitted foreign animal disease that threatens the health and economy of the U.S. equine industry. Previous outbreaks have caused significant economic losses and trade restrictions in the US. The ongoing outbreak in Florida highlights the critical need for early and accurate detection and diagnosis.
Current federally approved diagnostic procedures are labor-intensive, time-consuming, and variably successful. To address these limitations, we identified three novel TE-specific gene targets through comparative genomic analysis. This study aims to develop and evaluate a digital PCR (dPCR) assay for species-specific TE detection.

METHODS:
Oligonucleotide sets (primers and probes) for selected three species-specific gene targets were designed and tested in singleplex and multiplex dPCR assays using target-specific plasmids (pUC57) as positive amplification controls (PACs). Specificity and sensitivity were determined using serially diluted PACs, gDNA extracted from 13 epidemiologically distinct TE strains, closely related *T. asinigenitalis* (TA), and 12 non-TE bacteria.

RESULTS:
Two of three oligonucleotide sets showed high efficiency, consistently detecting all epidemiologically distinct TE strains while not detecting any non-TE strain. The results demonstrated that the duplex PCR results in species-specific detection of TE. Additionally, it differentiates TE from closely related TA isolates.

CONCLUSION:
The digital PCR assay offers species-specific, sensitive detection of TE, enhancing diagnostics for CEM. Research is ongoing for further optimization and validation of the multiplex dPCR assay for the detection of TE from equine genital swabs is ongoing with non-TE bacteria and spiked genital swabs from horses.

Investigating the role of Magel2 during nutritional stress using single nucleus RNA sequencing

Denis Štepihar,[1,2] Christian Bustamante[3], Maria Hoyos Sanchez[1,2], Tara Bayat[1,2], Farzana Popy[1,2], Isabel Castro[3], Sambantham Shanmugam[3], Jorge Riano Diaz[1,2], Igor Ponomarev[3], Stephanie Myers[1,2], and Klementina Fon Tacer[1,2]

Institution(s):

[1] School of Veterinary Medicine, Texas Tech University, 7671 Evans Dr., Amarillo, TX [2]Texas Center for Comparative Cancer Research (TC3R), 7671 Evans Dr., Amarillo, TX [3]Texas Tech University Health Sciences Center, 3601 4th St, Lubbock, TX

Abstract:

Objective:

The MAGE family protein MAGEL2 is involved in Prader-Willi (PWS) and Schaaf-Yang (SYS) Syndromes, which are rare, genetic, multisystemic, neurodevelopmental disorder. The hallmark of both symptoms is uncontrolled satiety. On a cellular and physiological level, MAGEL2 is associated with protein transport and hypothalamic neuroendocrine function, respectively. Several MAGE gene family members were shown to protect cells from stress and allow for faster adaptation to changes in environment, but the role of MAGEL2 during stress is not known. **Methods:** To address the role of MAGEL2 during fasting, we exposed wild-type and *Magel2*$^{p\Delta/m+}$ (KO) male mice to 24h-fasting-induced stress (4 animals per group). Then we applied 10X technology to perform single nucleus RNA sequencing (snRNAseq) analysis of hypothalami, 2 animals per group we pooled for snRNAseq analysis. Data was analyzed with CellRanger (v7.2.0) and Seurat (v5.0.3). Based on the established cell type markers, we classified 23 cell types. Then, we analyzed cellular composition and performed differential gene expression (DEG) analysis.

Results and Conclusions: We found that while the ratio of cell types did not change significantly between the different groups, their transcriptomes were altered. The highest number of DEGs upon starvation and Magel2 depletion were observed in non-neuronal cells, such as microglia, tanycytes, endothelial cells, and oligodendrocyte precursor cells. Importantly, we discovered that the transcriptional profile of most cell types in fed *Magel2* KO mice resembled that of fasted wild-type mice, suggesting a role for Magel2 in regulating satiety.

Acknowledgements/Funding:

This work was supported by the Foundation for Prader–Willi Syndrome Research Grant 22-0321 and 23-0447 (to K.F.T. and Z.K.), Texas Tech University start-up (to K.F.T.), and Cancer Prevention and Research Institute of Texas Scholar Award RR200059 (to K.F.T.).

MAGEA3/6 Enable Pancreatic Cancer Cell Survival and Growth under Nutrient Stress

Sima Tozandehjani[1,2], Sara Uhan[1,2], Barbara Breznik[1,2,3], Juan Sebastian Solano [1,2], Jerneja Koren[1,2,3], Vesna Jurjevic[1,2,3], Miloš Vittori[1,2,4], and Klementina Fon Tacer[1,2]

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Introduction: Melanoma-associated antigens (MAGEs) are implicated in aggressive disease progression and treatment resistance across multiple cancer types. However, their role in pancreatic cancer remains poorly characterized. Our preliminary analysis of The Cancer Genome Atlas (TCGA) data revealed that 10% of pancreatic tumors express MAGEA3/6 and promote resistance to glycolysis inhibition, suggesting their role in pathogenesis of this devastating disease. This study seeks to elucidate the molecular mechanisms by which MAGEA3/6 influence pancreatic cancer cell metabolism and disease progression. **Methods**: To investigate the roles of MAGEA3/6, we overexpressed these genes in highly glycolytic MIA PaCa-2 pancreatic cancer cells. Cells were subjected to metabolic stress, including glycolysis inhibition using 2-deoxy-D-glucose (2DG) and glutamine deprivation. Viability and metabolism were assessed using viability (Alamar Blue) and metabolic flux (Seahorse) analysis, transmission electron microscopy (TEM), and RNA sequencing. Validation was performed by Western blotting (WB) and immunofluorescence microscopy. **Results:** MAGEA6-expressing cells demonstrated resistance to 2DG-induced glycolysis inhibition but heightened susceptibility to glutamine depletion. These cells showed increased ATP production and mitochondrial oxidative phosphorylation under 2DG stress. TEM revealed distinct mitochondrial changes, corroborated by Mitotracker dye intensity in MAGEA6-expressing cells under stress. WB analysis indicated reduced hexokinase (HK) expression in MAGEA6 cells following 2DG treatment. RNA sequencing highlighted significant transcriptomic alterations, including upregulated MAPK pathway. **Conclusion:** Our data suggest that MAGEA3/6 enable pancreatic cancer cell growth under metabolic stress by enhancing mitochondrial function and activating MAPK . Targeting MAGEA3/6 alongside metabolic and signaling pathways may offer therapeutic potential for patients with MAGEA3/6-positive tumors.

Impact of Antineoplastic Drugs on Gap Junction-Mediated Cell Communication in Co-Cultured Colorectal Cancer Spheroids

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Recent advancements in colorectal cancer research have highlighted the critical role of the tumor microenvironment. A key component of this microenvironment is fibroblasts which influence drug response and resistance. Evidence indicates that gap junctions are often dysfunctional in colorectal cancer, leading to reduced drug efficacy. Restoring gap junction functionality has the potential to enhance the effectiveness of antineoplastic agents. This study aims to use co-culture spheroids and evaluate how fibroblasts and gap junction restoration impact the efficacy of antineoplastic agents. Methodology involves creating co-culture spheroids by combining fibroblast cells (CCD-112CoN) and colorectal cancer cells (SW620, SW480, HT-29, or Caco-2) in a 1:1 ratio. Two-week cultured spheroids were filtered to achieve uniform sizes. Individual spheroids were treated with antineoplastic drugs in a 96-well plate. Four drugs (doxorubicin, erlotinib, nilotinib, and cediranib) were tested at six different concentrations: 500 nM, 100 nM, 50 nM, 10 nM, 1 µM, and 10 µM. Additionally, the synergistic effect of antineoplastic drugs was tested in combination with a gap junction enhancer. Cell viability was assessed using MT Cell Viability Assay. The results revealed that LD50 for doxorubicin in Caco-2 spheroids at 24 hours was 500 nM, whereas in co-culture spheroids, most cells died at this concentration. Moreover,

the addition of PQ compound reduced LD50 in Caco-2 spheroids to 50 nM compared to 100 nM doxorubicin alone. These findings suggest an interaction between antineoplastic drugs and fibroblast cells. In conclusion, these results highlight the potential of using co-culture spheroid models to investigate drug efficacy in colorectal cancer.

Comparative phylogenomics of *beta-lactamase*-producing *Escherichia coli* at one health interface.

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Abstract

Objective: In recent years, many significant reports reported about the spreading of resistance and bacterial potential to transmit mobile genetic elements that give resistance to many drugs. We aimed to determine the genetic relatedness among beta-lactamase-producing *Escherichia coli* isolated from soil, lake water, and feces of geese, pigs, dogs, cattle, coyotes, wild-hogs, and horses at one health interface in West Texas.

Methods: 65 beta-lactamase-producing *E. coli* isolates were included in this study, previously isolated using ESBL chromogenic agar. These isolates were previously recovered from the feces of geese(n=13), pigs(n=12), horses(n=10), coyotes(n=4), dogs(n=13), cattle(n=6) and wild-hogs(n=1), and environmental samples (soil(n=5) and water(n=1) from recreational parks) from different locations in West Texas. Genetic relatedness between the isolates was determined using whole-genome sequencing (WGS), core genome multilocus sequence typing (cgMLST), and phylogenetic single nucleotide polymorphism (SNPs) analysis using the publicly available bioinformatic platforms.

Results: Among 65 beta-lactamase-producing *E. coli* isolates 47 isolates were carrying *bla*CTX-M genes including *bla*CTX-M-1(n=11),*bla*CTX-M-55(n=10),*bla*CTX-M-15(n=13),*bla*CTX-M-65(n=5),*bla*CTX-M- 27(n=4),*bla*CTX-M-32(n=3),*bla*CTX-M-14(n=1), 25 isolates were carrying *bla*TEM genes including *bla*TEM-1A,*bla*TEM1B,*bla*TEM-141,*bla*TEM-206,*bla*TEM209,*bla*TEM214, 6 isolates contained *bla*OXA-10, and *bla*CMY-2 gene. The predominant phylogroups were B1(n=22), and A(n= 14). Among diverse serogroups and sequence types identified, O101:H9(n=4) and ST10(n=5) were mostly found in isolates from pigs. Based on cgMLST, 34 clonal lineages were identified. By using 100 maximum number of SNPs between the isolates to define a clone, 13 clones were identified in 17 isolates. 48 isolates were identified as multidrug-resistant.

Conclusion: The findings from this study provide limited evidence of clonal dissemination of beta-lactamase-producing *E. coli* between different animal sources.

Maternal Pea Fiber Supplementation Improves Energy Balance, Glycemic control and Cardiac Function in Spontaneously Hypertensive Stroke-Prone Rats and Their Offspring

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Background: Previous rodent studies showed that maternal diets high in inulin fiber protect the offspring against obesity. However, the specific effects of maternal pea fiber on energy balance and cardiovascular health remain underexplored. We determined the effect of maternal pea fiber (PF) supplementation on energy balance, body composition, glucose clearance, and indices of cardiac function, in dams and offsprings of Spontaneously Hypertensive Stroke-Prone (SHRSP) rats. **Methods:** Female SHRSP rats (n=44, 8-10 weeks old) were fed a high-fat-diet (4.76 kcal/g) for 3-weeks, bred, and randomized to: Control (C), Pea Fiber (PF, 25% wt/wt, during gestation and lactation, control diet postweaning) and Control+Pea Fiber (C+PF, pea fiber during lactation and postweaning). Food intake, energy expenditure (EE), respiratory quotient (RQ), body composition, glucose tolerance, and echocardiography were assessed. **Results: Maternal:** Compared to C, the PF reduced RQ and EE during gestation and lactation, and reduced fat mass in lactation. C+PF increased lean mass and enhanced blood glucose clearance in lactation. **Offspring:** PF-offspring showed reduced RQ, C+PF male offspring had reduced RQ and fat mass, and female offspring had reduced weight and fat mass. PF and C+PF offspring had higher ejection fraction rates and stroke volumes at weaning. **Conclusion:** Maternal supplementation of pea fiber increased fat utilization, reduced adiposity, and improved glycemic control in dams, and part of these benefits were imprinted on their offspring. Postnatal pea fiber supplementation reduced adiposity and improved cardiac function in adulthood.

Extracellular vesicles isolation and characterization approaches from bovine colostrum and its impacts in neonatal dairy calves' immune system

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OBJECTIVE: Extracellular vesicles (EVs) are membrane-bound particles naturally released from almost all types of cells in the mammalian body. Such EVs have different cargo that might regulate different metabolic and immune functions. This study aimed to characterize EVs derived from bovine colostrum.

METHODS: Colostrum samples (n = 6) were collected from Holstein dairy cows (Hereford, TX), defatted, and EVs were isolated by ultracentrifugation. Dynamic light scattering measurements were performed to check size distribution (Zetasizer Nano ZS90, Malvern). Further, EVs isolates were subjected to Transmission Electron Microscopy (EM) and Cryo-Electron Microscopy (Cryo-EM). Extracellular vesicles from colostrum were also captured on Luni chips loaded with bovine specific anti-CD9 and anti-CD63 for single particle interferometric reflectance imaging on a Leprechaun instrument (Unchained Labs, Pleasanton, CA, US).

RESULTS: The EM analyses confirmed the colostral EVs to fall within the range of 40-150 nm. The Cryo- EM analyses showed our isolated EVs as nanoparticles with clear lipid bilayer boundaries, and with a donut shape. Mean diameter of ~ 45 nm and particle concentration within a 2-fold range across the EV samples were detected. Additionally, we detected CD63 and CD9-bound EVs, in both defatted colostrum samples, and in EVs isolated by ultracentrifugation.

CONCLUSIONS: These findings suggest that although the colostrum heterogeneity presents a challenge for EVs isolation, applications of these methods potentially contribute to the characterization of EVs from bovine colostrum. Our findings enhance our understanding about bovine milk EVs while more research is needed to evaluate the impact of Colostral EVs cargo on the offspring.

Foodborne Pathogen Management: Targeting different *Salmonella* serovars with Acid-Producing Bacteria Combinations

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Objective: *Salmonella* contamination is a critical challenge in the meat industry, threatening food safety and public health. Effective control measures are essential. Acid-producing bacteria (APB) offer promising pre- and post-harvest interventions. This study aimed to determine the antagonistic effects of APB combinations by measuring their effectiveness in inhibiting *Salmonella* serovars.

Methods: Eleven combinations of *Lactiplantibacillus plantarum* (A), *Bifidobacterium bifidum* (B), *Propionibacterium freudenreichii* (C), and *Propionibacterium jensenii* (D) were prepared and the antimicrobial effects against *Salmonella* serovars (Enteritidis, Typhimurium, Kentucky, Newport, Dublin, Heidelberg, Infantis) were assessed using an agar-well diffusion assay. After overnight growth, each *Salmonella* culture was swabbed onto Mueller-Hinton agar to create a lawn. Wells were punched in the agar and filled with 100μL of each combination culture. Gentamicin was used as the positive control. After 24-hour incubation, inhibition zones were measured. Data was analyzed by two-way ANOVA using GraphPad Prism.

Results: Antimicrobial activity was strongest in the four-APB mix, with inhibition zones averaging 29 mm, significantly outperforming gentamicin for *S*. Newport and *S*. Dublin ($P<0.05$). Combination C+D also exceeded gentamicin against *S*. Infantis and *S*. Newport. Combinations A+C+D and A+B+D strongly inhibited *S*. Dublin. Notable inhibition of *S*. Kentucky and *S*. Heidelberg was observed with B+C+D and B+C, while *S*. Typhimurium showed moderate susceptibility across treatments. Statistically significant differences in inhibition zones were observed across combinations and serovars, highlighting the potential of APB combinations to enhance S*almonella* control beyond standard treatments.

Conclusions: APB combinations significantly reduced *Salmonella* populations, with complete inhibition of multiple serovars, demonstrating effective pathogen control.

Public Perceptions of Calf Disbudding Techniques Used on Texas Farms

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Abstract

Research emphasizes the importance of understanding public opinion on farming practices. This study investigated Texan consumers' perceptions surrounding two calf disbudding methods: caustic paste (CP) and hot iron (HI) and their potential implications for animal welfare. A cross-sectional survey, consisting of a 44-item questionnaire, was distributed online to 514 participants across Texas using Qualtrics® and Centiment. The questionnaire collected data on demographics, dietary habits, familiarity with agricultural practices, and responses to visual images of disbudding methods. Statistical analyses, including chi-square tests, binary logistic regression, and t-tests, were conducted. The results showed that most participants (72.8%) preferred CP, associating it with better animal welfare. Gender and education had a strong influence on preferences, with females and more highly educated individuals favoring CP. Visual exposure to disbudding procedures, particularly HI, markedly, decreased participants' willingness to purchase, consume, or serve beef. Dietary habits, such as seafood and cheese consumption, also predicted a preference for CP. Exposure to scientific information fostered a shift toward more welfare-conscious opinions. Consumers perceive CP as more humane than HI, although observing either method negatively impacts purchasing behavior. These findings highlight the importance of education, transparency, and adopting welfare-oriented practices in the dairy industry. Exploring alternatives such as polled genetics and engaging consumers in decision-making can help align industry practices with public expectations.

Fecal microbiome of horses under different housing environments in West Texas

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Objective: This study aimed to describe and evaluate the effect of sex, breed, and different housing environments on the fecal microbial communities of healthy horses in West Texas.

Methods: Fresh fecal samples were collected from 70 horses at six locations, including rescue centers, equine centers, farms, veterinary clinics, boarding stables, and households. Data were gathered on sex, breed, work type, premises, and housing conditions. Fecal microbiota composition was analyzed using 16S rRNA sequencing on the Illumina MiSeq platform. Alpha and beta diversity, PERMANOVA, and SIMPER analyses were used to assess microbial community structure.

Results: Proteobacteria (51.39%), Bacteroidetes (26.73%), Firmicutes (17.45%), and Actinobacteria (4.08%) were the predominant phyla, While Microbacter (12.46%), Acinetobacter (6.85%) and Pseudomonas (6.25%) were the predominant genera among the horses. Significant differences in Actinobacteria abundance were observed across locations, breeds, sex, premises, work types, and housing conditions, while Bacteroidetes and Chloroflexi varied by location and breed, respectively. No significant differences in alpha diversity were detected among these factors, but beta diversity varied significantly by location, breed, premises, and housing ($p<0.05$). SIMPER analysis identified 21 operational taxonomic units (OTUs) driving differences by location, 6 OTUs by breed, and 1 OTU by premises. Multivariable PERMANOVA revealed that breed, premises, and housing conditions explained 61% of microbial diversity variation, with a significant interaction between work types and locations ($p=0.006$).

Conclusions: Both intrinsic and extrinsic factors significantly influence the fecal microbiome of horses. This information should be considered in the disease management of horses especially gut dysbiosis and other metabolic disorders.

The Quantification of *Myo*-Inositol in Swine

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The gestational and weaning phases in the swine industry involve multiple stressors that can disrupt normal physiological function and homeostasis when transitioning to new environments and farrowing. Two similar studies were performed involving Alginate Hydrogel Beads, infused with myo-inositol and electrolytes provided as supplementation during the transitional phases. In the past, Alginate Hydrogel Beads have had some promising effects on the outcome of swine stressors, however, the quantification of their effects on swine physiology remains unexplored. The objective of this study was to explore and quantify the amount of myo-inositol present in swine plasma and brain tissue of swine supplemented with myo-inositol, to make connections relating to overall performance, behavior, and physiological function. The study design was randomized with pigs in treatment, negative control, and positive control groups to determine if the supplementation vs control had significant performance changes. Blood plasma samples and brains were obtained for quantification analysis. The brains were homogenized using Lysis Buffer through sonication. Samples were analyzed via Liquid Chromatography-Mass Spectrometry (LC-MS). LC-MS data revealed group-specific differences, potentially influenced by factors such as sex, diet, and gestational nutrition in sows. These findings provide valuable insights into the physiological impacts of providing myo-inositol supplementation to swine during the most vulnerable phases, as a way to mitigate negative impacts on homeostasis and physiological function.

Keywords: *Myo*-Inositol, Quantification, Physiological

Authenticity of Cinnamon Verum Determined by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF MS) and Machine Learning

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ABSTRACT

Introduction: Cinnamon is the most popular spice globally and has several potential medicinal applications. *Cinnamomum verum* is the most valuable and is called ‘true cinnamon’ and is mainly produced in Sri Lanka. Other cinnamon spices, such as *Cinnamomum cassia*, are commercialized, are low-cost, and contain more hepatotoxic compound coumarin. On the other hand, *C. verum* includes a high concentration of antioxidant eugenol; however, the higher price makes it more vulnerable to adulteration, mainly through the addition of products of the same species, and it's too difficult and time-consuming to identify the adulteration by conventional methods.

Purpose: This study investigates whether MALDI-TOF-MS, combined with machine learning algorithms, is an easy, rapid, and cost-effective analytical method for the authentication of cinnamon.

Methods: Cinnamon samples were extracted using a solvent and spotted on a MALDI plate. Machine learning algorithms have been used to detect patterns embedded within MALDI-TOF spectra using a training set of 384 spots. Machine learning models such as decision trees, random forests, and gradient-boosted trees were used to classify a different scoring set consisting of 328 sample spots. The approach's sensitivity (*sens*) and specificity (*spc*) have been evaluated.

Results: After analyses of different machine learning models, the random forest model gave the best results, with a maximal Youden index of J = 1 achieved for the given set of conditions. Gradient-boosted tree and decision tree models also gave outstanding results with a maximal Youden index of J = 0.99 and 0.98, respectively.

Significance: We conclude that MALDI TOF MS, combined with the machine learning model, is a valuable and high-throughput tool for the authentication of cinnamon. This rapid and easy approach can prevent valuable food adulteration in the market and improve food safety too.

Melanoma antigen genes in testicular physiology and cancer: Protective roles on male germ cells during stress and immune response

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Melanoma antigens (MAGEs) are a subset of cancer-testis antigens (CTAs), which are normally expressed in the male germ cells of the testes known as immune-privileged sites of the body and subjected to specific physiological functions in reproduction and species survival. MAGE genes are aberrantly expressed in different cancers, whereas normal expression in the testis is found in male germ cells at different stages of spermatogenesis. They have been intensively studied for cancer therapy, however, the more fundamental questions about their physiological roles in the male germline remained neglected. To address this question, we have generated genetically modified mice with loss-of-function melanoma antigen (*Mage*) genes to get new insights into the physiological role of MAGE proteins. Our initial analysis of male animals suggested that Mages are dispensable for mouse fertility in laboratory conditions. Next, we exposed animals and germ cells to diverse stressors, e.g., DNA damage and calorie restriction, mimicking potential hazardous insults to male germline in a natural environment. We have found that in an un-optimal environment, Mages protected germ cells against stress and provided a reproductive advantage to males. Given the recent evolutionary expansion of the MAGE gene family in placental mammals, expression pattern of MAGEs, and phenotypes of *Mage* KO mice, we suggest that distinct MAGE genes evolved as an adaptation to diverse stressors. In our future work, we want to uncover the molecular mechanisms behind MAGE protective functions, their role in immune response, how they evolved in different mammalian species, and why they get hijacked in cancer.

Overcoming Language Barriers in Animal Welfare: Spanish for Specific Purposes Courses and Internship Practice with Veterinary and Animal Science Students in Spanish-Speaking Farm Settings.

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Language barriers between Spanish-speaking farmworkers and English-speaking veterinarians are a significant obstacle to effective communication, impacting animal welfare and farm productivity. This study addresses these challenges by integrating Spanish for Specific Purposes in Agriculture (SSPA) courses for veterinary and animal science students to improve their communication skills and animal welfare knowledge in Spanish-speaking farm environments. This program was a collaborative effort involving two additional institutions. Three SSPA courses were developed and delivered, followed by six-week on-farm internships, where students presented twelve training topics to farmworkers in Spanish. Student performance was evaluated through course scores, while farm workers' knowledge was assessed using pre- and post-intervention surveys and feedback collected through close-ended surveys. Data were analyzed using ANOVA to compare differences between pre- and post-intervention assessments across farms and topics, as well as regression analysis to evaluate the relationship between changes in knowledge and perceptions. The course completion rate was high (53.62%) compared to similar studies, despite challenges like the absence of course credits and academic workload. By the end of the intervention, 80% of Spanish speaking farm workers demonstrated improved understanding in topics such as Animal Health, Transport, and Behavior. Farm workers also provided positive feedback about the program's effectiveness and expressed enthusiasm learning from students. This study highlights that SSPA courses, combined with hands-on internships, and training provided in Spanish can effectively address language barriers on farms. Furthermore, they offer a scalable solution to enhance communication between veterinary professionals and Spanish-speaking farm workers, leading to improve farm productivity and welfare.

Meta-Analysis of Biofilm Production and Its Potential Link in the Successful Persistence of Non-typhoidal *Salmonella* in Food Chain

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Introduction: Non-typhoidal *Salmonella* (NTS) is a major cause of foodborne illness in the U.S., linked to various food sources. Biofilm production is crucial for NTS persistence throughout the food chain. However, inconsistencies in studies investigating NTS biofilms have hindered our understanding of links between the persistence of NTS, biofilm formation, serotypes, and sources. To address this knowledge gap, we conducted a meta-analysis and evaluated the association between these factors in NTS.

Method: A literature search was conducted using the keywords "*Salmonella*" and "biofilm" in PubMed, Scopus, and Web of Science. Articles meeting the selection criteria—utilizing at least one conventional assay (colony morphology, microtiter plate, tube, or calcofluor tests) and providing information on strains, sources, and serotypes—were included. From 109 selected studies biofilm characteristics of 6,112 strains representing 172 serotypes across four major sources were documented. Chi-square analysis was employed to evaluate associations among these factors

Result:

The prevalence of biofilm-positive strains showed an increasing prevalence from animals (75.65%; 1609/2127) to food (81.31%; 631/776) to humans (85.71%; 690/805). In contrast, the prevalence of biofilm-negative strains was in decreasing trend from animal (24.35%; 518/2127), food (18.69%; 145/776), and human (14.29%; 115/805). Furthermore, a significant association between serotypes and biofilm formation ($\chi^2 = 218.97$, $p < 2.2e\text{-}16$) was determined.

Conclusion: Biofilm-positive *Salmonella* prevalence increases from farm (animals) to fork (humans), suggesting that biofilm may pose a higher risk of persistence and transmission of NTS throughout the food chain. Variability in biofilm production across serotypes identifies high-risk strains, guiding targeted sanitation measures.

Development of Species Identification Keys of Hair Morphology of Mammals of Taiwan Using the Light Microscope and Scanning Electron Microscope

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Body:

OBJECTIVE: Hair is a characteristic feature of mammals. Its polymorphism, resistance to adverse conditions, and ease of collection make it ideal for species identification. The study of hair morphology is widely applied in forensic science, ecology, and archaeology. Beyond the use of light microscopes, the scanning electron microscope (SEM) featuring high resolution and depth of field has also been employed in trichomorphology. Despite the existence of many hair atlases and identification keys targeting mammals from different regions worldwide, a systematic analysis of mammalian hair morphology in Taiwan is lacking. The study aims to establish a hair database for terrestrial mammals of Taiwan and to construct an identification key by analyzing the characteristics of hair with optical microscopy and SEM.

METHODS: Quantitative and qualitative analyses of cuticle, medulla and other features of hair using the light microscope were conducted on dorsalateral scapular guard hairs collected from 30 mammal species of Taiwan. SEM examination was also conducted in select species.

RESULTS: Quantitative features, including hair length, maximum width, and medullary index, as well as qualitative features like cuticular patterns and medullary patterns, exhibited varying degrees of phylogenetic relationships among species. Distinct morphology of the root, the base and the tip of hair were observed among species. Variations of the interscale distance along the hair also differed and may serve as a clue for identification.

CONCLUSIONS: Integrating these hair characteristics, we successfully established a hair morphology identification key for 30 terrestrial mammals of Taiwan, demonstrating a simple and cost-efficient method of species identification.

Anatomical angles of the rider influences Equine-Assisted Service horse gait symmetry

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Rider posture asymmetries can affect horses' movement during equine-assisted services (EAS); thus, we examined the influence of rider posture on horse gait during riding sessions across four EAS centers. Participant rider (n=53) posture was assessed using static images measuring shoulder tilt, trunk lean, and joint angles, while sensors on horses (n=22) measured gait parameters, including peak ground force, stride duration, symmetry, and length at the walk and trot. Results from general and mixed linear regression models show that rider left trunk lean positively influenced the peak ground reaction force in the horse's left front limb at the walk (P=0.03). At the walk, rider left knee angle improved horse left hind stride symmetry (P<0.005), while right hind stride symmetry was positively affected by left hip angle (P=0.01) but was negatively influenced by left knee (P=0.01) and right hip angles of the rider (P=0.04). At the trot, horse right front stride duration decreased with both left (P=0.02) and right shoulder angles of the rider (P=0.03) and left hind stride duration decreased with rider left shoulder angle (P=0.05). Right front stride symmetry increased with rider posterior trunk lean (P=0.04), while right hind stride symmetry decreased with rider shoulder and hip angles but increased with left knee (P<0.005) and right hip angles (P=0.01). Results suggest that rider posture influences horse movement, highlighting the need for further investigation into the influence of the rider on horse behavior and welfare during EAS sessions.

Decoding Diet: Uncovering Links Between Canine Nutrition and Gastrointestinal Health

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Previous research has examined specific dietary trends, such as grain-free, raw, probiotic diets, with one study identifying beef, dairy products, chicken, and wheat as the most common canine food allergens. Our study takes a broader approach,
analyzing a wide range of factors, including food types, preparation methods, and feeding frequencies to identify dietary patterns that contribute to gastrointestinal issues.

This research leverages data from the Morris Animal Foundation's Golden Retriever Lifetime Study (MAF-GLS), which tracked 3,000 dogs over seven years, to investigate the relationships between diet and gastrointestinal health, with a particular focus on diarrhea and vomiting. are linked to an increase in We utilized factor analysis to identify how dietary data, such as food types, preparation methods, and ingredient composition relate to gastrointestinal health. Structural equation modeling was used to evaluate how these dietary patterns influence the frequency of diarrhea and vomiting. Additionally, chi-square tests were conducted to compare observed versus expected frequencies of gastrointestinal issues across different dietary categories. All analyses were performed usingR and RStudio.

Study results aim to identify specific dietary patterns associated with an increased risk of gastrointestinal issues in dogs, offering actionable insights to improve dietary recommendations and canine welfare. Preliminary findings suggest that vegetables in the *Fabaceae* genus are linked to the highest frequency of diarrhea cases compared to vegetables in other genera.

Targeted Communication & Educational Training for Farm Animal Veterinarians and Students: Education to Enhance Feedlot/Dairy Adherence to Postmortem Procedures

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ABSTRACT

Postmortem examinations are a crucial diagnostic tool for disease management, surveillance and production management in food animal medicine. However, limited evidence exists on how often postmortem examinations are performed on farms. Reluctance in performing postmortem examinations is often linked to time restrictions, personal beliefs, lack of expertise and concerns surrounding potential exposure to zoonotic diseases. This study aims to identify and assess the beliefs, challenges, and limitations surrounding postmortem examinations in feedlots and dairy farms located around the Texas Panhandle and surrounding areas. The hypothesis is that addressing identified barriers through targeted training could improve the frequency and quality of postmortem procedures. We developed a semi-structure survey that captures key details, including job responsibilities, level of experience with postmortem procedures, training in personal protective equipment (PPE), use of digital technologies, reporting methods, perceived barriers, and recommendations for improvements. The questions contain multiple-choice questions, a Likert- scale assessment, and open-ended questions that could yield both quantitative and qualitative information. Analysis will follow a mixed methodology approach, combining descriptive and inferential analyses using R software (2024.12.0+467 version) with qualitative analysis conducted in NVivo 15 to identify recurring themes related to the practice and reporting of postmortem examinations. Preliminary pilot results will be presented and provide insight into the current practices, barriers, and training gaps associated with postmortem examinations in feedlots and dairy farms. The findings from this study will inform the development of targeted training programs aimed at addressing the identified barriers and improving the adoption of postmortem procedures.

Keywords: Postmortem examinations, communication skills, barriers, feedlots, dairy farms

Comparison of three diagnostic tests for arrival immunity status on future outcomes of beef-dairy calves.

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Adequate passive transfer is critical for the health and productivity of neonatal calves. Identifying calves with failure of passive transfer (FTP) remains suboptimal. The industry standard for identifying FTP is measuring serum total protein with Brix refractometers. The study objective was to compare three diagnostic devices: digital and analog Brix refractometers, and a commercially available immunity test (D2Dx™) to asses immunity status and predict future health events in day-old calves at a commercial operation in New Mexico. Serum from 534 calves was analyzed from newly received calves using the three devices. Corresponding health data was also collected throughout the in-hutch period. Serum data was compared using Kendall's tau correlation tests. Health events were evaluated with devices using logistic regression models. Receiver operating curve (ROC) analyses were performed to determine the predictability of future mortality and morbidity events.

There was a strong agreement between digital and analog Brix refractometers ($\tau = 0.78$, $P < 0.01$). D2Dx had a weak correlation with both analog and digital TP ($\tau = 0.05$, $P = 0.09$; respectively). D2Dx was found to be associated with both morbidity and mortality events ($P=0.04$ and $P<0.01$, respectively). ROC analyses for devices showed an AUC=0.57 for Brix TP and D2Dx for both morbidity and mortality events.

In conclusion, analog and digital Brix refractometer data were similar. D2Dx data was found to be associated with health events. However, no device was good at predicting future health outcomes. Further research is needed to develop better tools for identifying FTP and predicting health outcomes.

Assessing Ultrasound Probe Disinfection in Veterinary Clinics: A Hidden Risk for Bacterial Transmission?

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Objective: Ultrasound imaging is a non-invasive and cost-effective diagnostic tool that is widely employed in veterinary medicine where two types of ultrasound probes are routinely used: rectal and non-rectal probes. Bacterial contamination of ultrasound probes poses a serious risk of transmitting bacterial infectious agents between animals and from animals to humans (zoonoses). Use of properly disinfected probes is crucial to prevent such transmission, yet little is known about current practices in veterinary clinics. The objective of this study was to evaluate current probe disinfection procedures and the extent of bacterial contamination in veterinary settings.

Methods: Twenty-five veterinary clinics were recruited. Probe disinfection procedures at each clinic were recorded using a standardized questionnaire. Ultrasound probes *(n* = 60*)* were sampled to assess level of bacterial contamination via total aerobic bacteria and *Staphylococcus* spp bacterial culture.

Results: Nearly all probes (58/60, 96.67%) were contaminated, with 1.18 to 5.65 log10 CFU of total aerobic bacteria/probe. Additionally, 70% (42/60) of probes were contaminated with 1.18 to 3.56 log10 CFU of *Staphylococcus* spp./probe. Notably, the extent of bacterial contamination showed variation depending on the animal species treated by these clinics. While all clinics reported cleaning probes after each use, the procedures varied widely, and only two clinics had a written standard operating protocol (SOP) for disinfection.

Conclusions: These findings highlight the potential of ultrasound probes as vectors for bacterial transmission and underscore the need for the implementation of SOP for disinfection of probes in veterinary practices to minimize infection risks.

Ischemic Stroke Recovery of a Type 2 Diabetic Preclinical Female Model

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Abstract

Diabetes mellitus increases the prevalence of a variety of health issues, especially in the incidence of ischemic stroke. *Myo*-inositol, an organic osmolyte regulator, potentially assists with maintaining cellular edema following ischemic stroke. Prior studies have shown a decreased infarct and edema ratio following ischemic stroke in *myo*-inositol treated Type II Diabetic male mice models, however there is little known on female models. The present study aimed to evaluate *myo*-inositol's role on stroke outcomes in female diabetic and non-diabetic mice, and to compare with previously published studies on males. In vitro, immunocytochemistry on primary astrocytes was used to assess the *myo*-inositol transporters (SGLT6 and SMIT) and the sex differences between them. Transient middle cerebral artery occlusion (tMCAO) mouse model was used to evaluate the therapeutic efficiency of *myo*-inositol (30 mg/kg dose) using female non-diabetic (C57BL/6) and diabetic (B6.BKS) mice models. Vaginal lavages, blood glucose readings, and behavioral tests were analyzed on the mice throughout the study. In tMCAO, infarct and edema ratios following 3-hour and 24-hour reperfusion were decreased for *myo*-inositol-treated non- diabetics compared to non-treated non-diabetics. The *myo*-inositol-treated non-diabetics (ND w/ MI MCAO) had a decreased infarct and edema ratio compared to non-treated non-diabetics (ND MCAO). These results propose that *myo*-inositol may have neuroprotective qualities pertaining to ischemic stroke in both non-diabetics and diabetics.

Comparison of Simple Continuous Surgical Pattern versus Vest-Over-Pants Surgical Pattern for Linea Alba Closure in Cattle

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Body:

Incisional complications following a ventral midline celiotomy, although uncommon in the ruminant, can be a serious post-surgical complication. There is much debate in the veterinary field as to the ideal surgical pattern for closure of ruminant abdominal surgeries. Such studies have been conducted in the equine medical field, but there has been no adaptation into ruminant medicine. This study sought to compare Time-to-Failure (TtF) and Mode-of-Failure (MoF) of a ventral midline abdominal closure with either a simple continuous (SC) suture pattern or a vest-over-pants (modified-mayo) (VoP) interrupted pattern, both utilizing surgeon's start/end knots. The authors anticipated the SC pattern would both take less time to complete and hold up longer under pressure. 6 adolescent bovine cadavers were assigned to each experimental group. A 6.5-in ventral midline abdominal incision was made in each cadaver, and prior to closure a 100L inflatable air bladder was placed into the abdominal cavity. The professor then performed four closures per group, and the student performed two closures per group. The bladder was inflated at a steady rate until the first sign of failure of the hernia closure. Utilizing the data collected from both cohorts, no statistically significant differences were found between the TtF in each group; however, the SC pattern took significantly less time to complete, used less suture, and had a lower overall associated cost when compared to the VoP. Overall, the SC pattern is easier to perform for a practitioner of any level and more cost friendly, while not sacrificing efficacy.

Evaluating the impact of scratchers on fearfulness and claw health for cats in confinement

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Abstract Body:

Cats confined for veterinary and research use experience stress, which can be reduced with enrichment. This study's objective was to measure fearfulness in cats with/without scratchers and evaluate changes to claw health. Twelve cats participated; 6 given scratchers in their kennels which were replaced weekly after baseline testing (SCR; n=6), and 6 without (NO SCR; n=6) for 3 weeks. Weekly, cats participated in an open field test (10 min) and novel object test (5 min) in a grid-zoned room. In open field, vocalizations and zone changes were recorded. In novel object, time to contact the object was measured up to 5 minutes. After, cats had the front claws of digits 2-5 noted as either 'healthy' or 'chipped/cracked' via visual inspection. Behavior observations were done by a single observer. The number of zone changes was affected by time ($P < 0.001$) with the highest activity at baseline (53.2 ± 7.18) and no difference after (wk 1: 31.5 ± 7.18, wk 2: 32.2 ± 7.18, wk 3: 33.8 ± 7.18). Treatment did not affect the number of zone changes (SCR: 38.2 ± 9.30, NO SCR: 37.2 ± 9.30; $P = 0.94$). Six of the cats had claw issues during the study, and 4 of them were in NO SCR. Adding a scratcher did not modify tested behavior. Increased zone activity at baseline may be due to the novelty of the room at the start of the study. Also, providing a scratcher may benefit claw health, though further investigation is needed.

The use of a fixed visual reference point for determining veterinary students' ability to identify forelimb lameness.

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Body: Equine lameness identification is an essential component of equine practice. The correct identification of the lame leg and grade of lameness is imperative for proper diagnosis and treatment. Despite this skill's importance, a paucity of literature describes how to best teach veterinary students to identify lameness. This study hypothesizes that veterinary students will have greater accuracy at forelimb lameness identification when a fixed visual reference point is placed in the background during lameness exams. Institutional permission to use human subjects was granted (IRB2024-163). To test this hypothesis, 5 horses with forelimb lameness were video recorded while trotting during lameness exams. The background of the video recording was edited to a solid white background or a white background with gridlines. The resulting 10 recordings were randomly inserted into an on-line survey and distributed via ExamSoft to first- and second-year veterinary students(n=61). The students were asked to watch the video recordings and answer the following questions: is the horse lame, localization of the lameness and grade according to the AAEP standard? Students were also asked to provide their experience level with lameness prior to taking the survey. Responses were compared to evaluations by a board-certified equine surgeon. Statistical analysis using McNemar's and Wilcoxon Signed Rank tests revealed no overall improvement in diagnostic accuracy associated with the grid background. Resulting p-values (p1=0.58, p2=1, p3=0.53) suggest that background modifications may not uniformly enhance lameness identification accuracy. Future studies should explore larger sample sizes and alternative instructional tools to refine equine lameness training methodologies.

Establishing a Renewable Frozen Tissue Biobank: Enhancing Research Longevity and Sustainability

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In veterinary medicine, maintaining sample integrity and ensuring reproducible results are critical challenges in long-term storage; however, it is essential for preserving the quality and reliability of biological specimens for future research. This study aims to establish a novel solution for long-term biological sample storage by enabling researchers to maintain reliable samples over time. We investigated the viability and stability of variably-sized tissue samples processed either fresh, flash-frozen or slow-frozen with cryopreservation. Samples were compared through histology, western blot, PCR, RNA extraction, and cell culture to test cryopreservation effectiveness. Canine liver tissue from adult dogs was extracted using 2 mm and 8 mm punches. Slow-frozen tissues were placed in 1 mL of Cryostor media and slow-frozen until −80°C, flash-frozen tissues were frozen with liquid nitrogen, and fresh tissues were utilized immediately. All frozen tissues were stored at −150°C for one week. Preliminary findings show successful DNA extraction from fresh liver tissue, averaging 222 ng/µl with a 260/280 ratio of 2.04, and RNA extraction averaging 138 ng/µl with a 260/280 ratio of 1.98. The BCA assay confirmed successful protein extraction, with an average of 6.5 ug/ul for 8 mm samples and 8.7 ug/ul for 2 mm samples. We expect slow-frozen or cryopreserved tissues to be viable for all tests, while flash-frozen tissues will likely reflect cell death and fail viability tests. If cryopreservation at larger sample sizes proves effective, it could revolutionize tissue accessibility in research.

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Genotypic and phenotypic characterization of fecal beta-lactamase-producing *Citrobacter sedlakii* isolated from horses

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While there is the available information on the multi-drug resistance potential of *Citrobacter freundii* in horses, there is no information on the beta-lactam resistance dynamics of *Citrobacter sedlakii* in horses. In this study, we characterized phenotypically and genotypically nine *C. sedlakii* isolates from the feces of horses and then compared them to *C. sedlakii* isolates from humans using whole genome sequencing and phylogenomic methods. Seven isolates (7/9) were ampicillin-resistant, while at least one isolate was resistant to nalidixic acid, ceftriaxone, gentamicin, meropenem, and streptomycin. All the nine isolates were carriers of narrow- spectrum and chromosomal-mediated $bla_{\text{SED-1}}$ beta-lactamase gene which confer resistance to ampicillin. One isolate was positive for the *mcr-9* gene that confers resistance to colistin, and another isolate had the *aac(6')-lid* gene that confers resistance to aminoglycosides. Seven isolates (7/9) were carriers of genes that confer metal resistance to copper, silver, and arsenic. Five isolates (5/9) had plasmids with *IncFII* and *Col440I* most common, and seven (7/9) had transposons and insertion sequences with six isolates positive for both *Tn6292* and *IS26*. All isolates contained the lipoprotein *nlpI* precursor that supports bacterial adherence and invasion of intestinal epithelial cells. Similar genome analysis showed that eight isolates were genetically like human-derived *C. sedlakii* isolates previously reported from China, Japan, Singapore, and the United States. The results from our study provide insight into the antimicrobial susceptibility of *C. sedlakii* in horses which was previously lacking, and the specific beta-lactamase gene mediating resistance.

Measurement of urinary electrolytes in healthy goats and those with urolithiasis.

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Urolithiasis is a common, life-threatening emergency in small ruminants. While the exact cause is unknown, inherent metabolism of calcium, magnesium, and phosphorus may differ in affected animals. The condition may be exacerbated in animals fed diets high in concentrate. The purpose of this study was to determine the levels of calcium, magnesium, and phosphorus in the urine of healthy goats fed two different diets (hay versus grain) and goats with urolithiasis.

Healthy goats (hay, n=11; grain with hay, n=13) and goats with urolithiasis (n=12) served as subjects. Urine was retrieved through cystocentesis and stored at -80°C. Uroliths from affected goats were analyzed for composition at the Minnesota Urolith Center (University of Minnesota, St. Paul, MN). An AA-6300 Atomic Absorption Spectrophotometer (Shimadzu, Kyoto, Japan) was used to quantify urinary calcium and magnesium concentrations. Urinary phosphorous and creatinine were measured using an AU 480. (Texas Veterinary Medical Diagnostic Laboratory, Canyon, Texas). All data were analyzed using PRISM v10.2.2 (GraphPad, Boston, MA) and MetaboAnalyst 6.0 (www.MetaboAnalyst.ca) was used to create a PCA plot. Urinary creatinine was used to normalize electrolyte concentration. Depending upon the results of normality testing, a Kruskal-Wallis test or a one-way ANOVA were used to determine significance amongst groups.

Urinary calcium (p=0.0021) and creatinine (p=0.0061) were significantly different amongst groups whereas magnesium and phosphorous were not. In conclusion, the measurement of urinary calcium was different amongst goats with urolithiasis. Further studies may indicate if urinary calcium could be used as a screening tool in goats for the development of urolithiasis.

Effects of Myo-Inositol on Energy Balance in Female Rats.

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Affiliations: Texas Tech University School of Veterinary Medicine Funding: Texas Tech University School of Veterinary Medicine

Abstract:

Background: Metabolic diseases including obesity, diabetes and hypertension severely strain our healthcare system. Myo-inositol (MYO) is a natural cyclitol isoform found in many foods in the typical American diet that has been used as a therapeutic for polycystic ovary syndrome and gestational diabetes. However, whether MYO improves energy balance and protects against obesity and hypertension is poorly understood. ***Objectives:*** We determined the effects of MYO on energy balance and blood pressure (BP) in female spontaneously hypertensive stroke-prone rats (SHRSP). ***Methods:*** Obesity was induced with a high-fat diet (HFD) in SHRSP rats (n=28). Rats were randomized into three groups: control (CON, n=10), 2% myo-inositol (2% MYO, n=9), and 4% myo-inositol (4% MYO, n=9) with MYO provided ad libitum in drinking water. Measurements included weekly blood pressure, MRI body composition, and daily indirect calorimetry (CLAMS®). ***Results:*** A transient decrease in food consumption was found in both 2% MYO and 4% MYO groups in during the first 2 days of the study compared to CON. Similarly, a transient increase was seen in the respiratory energy ratio (RER) with both 2% MYO and 4% MYO groups compared to CON. ***Conclusions:*** MYO improved energy balance by inhibiting food intake, shifting substrate use towards lipids, and transiently decreasing percent adiposity in female SHRSP rats. MYO effects on energy balance persist under fasting conditions but appear to be ineffective against genetic hypertension. The neurohumoral mechanisms by which MYO regulates energy balance and cardiovascular function, and the therapeutic potential of MYO against maternal obesity and hypertension remain elusive.

qPCR evaluation of the fecal microbiome in healthy horses and those with colic

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Colic is the most common form of gastrointestinal (GI) disease and is the leading cause of equine mortality. Recent evidence suggests that the GI microbiome is critical in equine nutrition, metabolism, and immunity and may contribute to equine colic. Next-generation sequencing has indicated that horses with colic experience dysbiosis or changes in the bacterial composition of the hindgut. While useful as a discovery tool, sequencing-based methods are expensive, time- consuming, and inappropriate for routine clinical use. This project aimed to develop an accurate, rapid, cost-effective molecular method to quantify the bacterial taxa in equine feces. Feces were collected from 20 horses (n=10, healthy; n=10, colic), and DNA was extracted using a PowerSoil® Pro (Carlsbad, CA) kit. Quantitative PCR assays were used to quantify the number of total bacteria (Universal) and 7 of the following bacterial taxa: *Blautia*, *Escherichia coli*, *Lachnospiraceae*, *Ruminococcaceae*, *Fusobacterium*, *Streptococcus*, and *Turicibacter*. A Mann- Whitney test (PRISM v10, GraphPad, Boston, MA) was used to determine differences between groups (significance set at $p<0.05$). *Streptococcus* ($p=0.0092$), *Fusobacterium* ($p=0.064$), and *Blautia* ($P=0.0601$) were significantly different between healthy horses and those with colic.

These results confirm that horses with colic have changes in the bacterial composition of the hindgut compared to healthy horses.

Flipped Classroom vs. In-Person Lab Demo to Improve Veterinary Students Short-Term Retention

Hailee Conley & Philippa Gibbons
Texas Tech University School of Veterinary Medicine

The flipped classroom (FC) is a method of teaching that flips the traditional classroom technique by giving online material before lab and using class time for discussion of the material.

Currently, most research focuses on participant satisfaction with the FC concept, and there is limited research on its effects on skill retention in veterinary education. The purpose of this study was to compare the effects of a FC versus the traditional method of in-class demonstration on short-term retention of how to place a cast on a canine leg.

This study examined the usage of a FC versus the traditional method by using two cohorts of professional veterinary students. Over 90 minutes, the traditional cohort received an in-lab demonstration on cast placement and then practiced placing one along with discussions. The flipped cohort received a 13-minute video demonstration and a 4-question quiz before the lab, followed by practicing cast placement with guided facilitation over 90 minutes. An objective structured clinical examination (OSCE) assessed the short-term retention of both cohorts.

Afterward, participants took a survey to evaluate if the materials given to each cohort were helpful and if they were satisfied with the study.

Out of the 184 veterinary students recruited, there were 18 participants. The mean on the pre-lab quiz taken by the flipped cohort was 91.8%. The OSCE scores for the flipped classroom and the traditional method were 4.2 and 4.1, respectively ($p<0.05$).

In conclusion, the FC did not significantly outperform the traditional classroom in this small study.

Impact of colostrum deprivation on ileal epithelia markers and inflammatory genes in beef-on-dairy calves

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OBJECTIVE: Colostrum consumption is crucial for improving the intestinal health of calves and reducing the incidence of gut diseases. This study aimed to assess the gene expression of tight junction proteins and inflammatory genes in the ileum of neonatal beef-on-dairy calves (n=24).

METHODS: Calves were randomly assigned to either a colostrum-deprived control group (n=12) or a colostrum treatment group (n=12), with the latter receiving 2.8 L of pooled colostrum via oral gastric tube within 6 hours after birth. Colostrum quality was 26% on the Brix scale. At 9 days of age, 6 calves in each group were inoculated with *Salmonella Typhimurium*. Inoculated calves were harvested, and intestinal samples were collected at 72h post-inoculation, while the non-inoculated calves were harvested at 23 days of age. Ileum tissue samples were collected from all calves for total RNA extraction and gene expression analysis via RT-qPCR. Genes of interest included specific intestinal epithelial markers (KRT8, FABP2), tight junction proteins (TJP1, CLDN1, CLDN4), and inflammatory markers (IL6, TNFA, NFKB, IL10).

RESULTS: At 72h post-inoculation, the treatment-inoculated calves had a tendency (P=0.07) for greater *CLDN1* expression relative to the control-inoculated calves. At 23 days of age, the treatment group had a tendency (P=0.07) for greater *NFKB* expression compared with the control group. No statistical differences were observed for the other inflammatory markers measured in the ileum of the calves.

CONCLUSIONS: Our results suggest a positive relationship between colostrum consumption and the intestinal health of beef-on-dairy calves during inflammatory conditions.

Postmortem onset and progression of rigor mortis and changes in body temperature in calves and dogs

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After death, the body temperature will adjust to the environmental temperature and the skeletal muscles will enter rigor mortis. Studying the timing of these changes can help estimate the postmortem interval which could be of central importance in cruelty investigations. This project studied the changes in body temperature and the onset/resolution of rigor mortis in calves and dogs after death. Thirty calves and 24 dogs were freshly euthanized for unrelated reasons to this project and kept at 24°C for 5 days. During this time, body temperatures were read every 2 - 4h, using a rectal/meat thermometer for the rectal temperature; an ear thermometer for ear canal temperatures and a no-contact thermometer read temperatures of the outer ear, forehead, nose, front paw pad and perianal skin. Concurrently, rigor mortis was evaluated on the jaw, neck, front limb, hind limb and tail. In dogs and calves, the rectal temperature decreased to equate the environmental temperature within 22-25h and 10-12h respectively; and the ear thermometer was unable to read body temperature (<32°C) after 10 and 4 hours respectively. All the no-contact measurements decreased to the environmental temperature within 6h, with the perianal skin temperature decreasing the slowest. Rigor mortis started in the jaw, progressed to the neck, hindlimb, then forelimb and was at its maximum after 6h. The tail never entered full rigor in calves and occurred last in some dogs. Rigor reversed in the exact opposite order and regressed the most by 30h in dogs, and 50h in calves.

The use of transcutaneous ultrasound to assess the gastrointestinal motility at 3 sites of the ascending colon.

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Reduced gastrointestinal motility may be a cause of impaction colics with anatomic locations such as the sternal and pelvic flexures and cecum commonly affected. Diet can play a significant role in influencing motility in horses. The purpose of this study was to investigate the effects of two diets on gastrointestinal motility at 3 anatomic locations commonly affected by impaction colic. Horses (n=12, Quarter Horses; median age 15 years, range, 5-25 years old; 7 mares and 5geldings) from the teaching herd at Texas Tech School of Veterinary Medicine served as subjects. Using a cross-over study design, horses were fed a complete pelleted ration (Purina Equine Senior fed at 1.35% body weight/day) and a forage-based diet (coastal Bermuda grass hay fed ad libitum supplemented with 0.5% body weight/day of Equine Senior). Horses were allowed to acclimate to assigned diets on study days 1-10. Fresh water was provided at all times. Gastrointestinal motility was measured on days 11-13 using a GE Logiq ultrasound with a curvilinear probe. Gain and depth were adjusted as needed. Horses were randomly assigned two time points (2 hours post-feeding) for measurement. Two ultrasonographers monitored and recorded gastrointestinal contractions in real-time at 3 locations (the sternal flexure, pelvic flexure, and cecum) for four consecutive, 1-minute periods. A contraction was defined as any movement of the large intestinal wall greater than or equal to 2 cm in magnitude. Data concluded that the sternal flexure motility was improved significantly on a grain diet (before diet change: P=0.00151, after diet change: P=0.000208).

The use of a fixed visual reference point in determining veterinary students' ability to identify hindlimb lameness in horses.

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School of Veterinary Medicine, Texas Tech University, Amarillo, Texas

Lameness detection is a crucial skill needed in equine practice, however, there is little documentation in the literature regarding how to best teach lameness identification. The purpose of this study was to determine if a fixed visual reference point, present in the background during lameness examinations, could improve the accuracy of veterinary students when assessing lameness in horses. Institutional permission to use human subjects was granted (IRB2024-163). Video recordings of 5 horses with hindlimb lameness were utilized. The backgrounds of these recordings were altered to either a solid white backdrop or a white backdrop with grid lines. The 10 modified videos were then randomly uploaded into a survey administered via ExamSoft.

Veterinary students (n=61) were asked to disclose their prior experience with identifying lame horses and then answer three specific questions: is the horse is lame, which limb is lame, and grade the lameness according to the AAEP standard. Subsequently, student responses were compared with those provided by the board-certified equine surgeon who examined the horses for lameness. Statistical analysis of was performed using a paired t-test in PRISM (Graph Pad, Boston, MA). The background had no effect on the students' ability to answer the three questions ($p > 0.05$). The data did however trend toward the grid background having an increasing accuracy for hindlimb lameness detection but did not reach significance. These findings provide insights into effective educational strategies for enhancing lameness identification skills among veterinary students.

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Hematological parameters in beef-on-dairy calves induced to systemic inflammation during the neonatal period

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OBJECTIVE: A complete blood count (CBC) analysis is a blood test that can provide insight into the overall health of an individual. While there are established reference intervals (RI) for adult cattle, there are no universal RI for young calves. This study aimed to evaluate the pattern of blood markers of inflammation in beef-on-dairy calves challenged with *Salmonella*.

METHODS: Beef-on-dairy calves (n = 24) were enrolled in this study from birth until 21 days of age. Twelve calves received colostrum at the first feeding (treatment group), while the other half were given milk replacer at their first feeding (control group). All calves were subsequently fed milk-replacer. At 8 days of age, 6 calves from each group were orally inoculated with *Salmonella Typhimurium*. Blood samples were collected prior to the morning feeding from the jugular vein into K2 EDTA tubes for CBC analysis.

RESULTS: Calves inoculated with *Salmonella* had higher neutrophils count and these numbers were higher than the RI for adult cattle. The total number of lymphocytes decreased after 9 days of age in all calves, except for the treatment non-Salmonella group. Blood monocytes were elevated in the blood of all calves during *Salmonella* infection. At 21 days, red blood cells count was elevated in the control group relative to the adult bovine RI.

CONCLUSIONS: This study highlighted the differences in the hematological parameters of neonatal calves relative to adult cattle, emphasizing the need to establish specific RIs for calves since calves have distinct physiological characteristics compared to adult cattle.

Equine melanoma: Development of *in vitro* models for therapeutic and mechanistic studies

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Equine melanoma occurs in an estimated 80% of grey horses. The purpose of our work was to develop *in vitro* cellular models for future therapeutic studies. Primary cell cultures developed from equine melanoma tissues were grown in various cell media (RPMI, EMEM, DMEM, and DMEM F12) supplemented with 10% fetal bovine serum (FBS) to determine optimal growth conditions. Fresh tissue was washed in cold PBS supplemented with antibiotics, cut into small pieces, and subjected to enzymatic digestion, including trypsin and collagenase. Digestion was performed at 37 C in a water bath or using an OctoDissociator (Myltenyi) with regular mixing for 30 minutes. Enzymatic digestion was stopped with FBS-supplemented media, and cells were repeatedly washed and plated onto 10 cm plates. To determine if cell lines can be isolated from frozen tissue, small pieces of tissue were frozen in RPMI media supplemented with 5% DMSO. Established primary cells were also tested for 3D growth using Celvivo 3D Cell culture technology. Cell lines that showed pronounced proliferation and organoid formation were then plated on Biolog pre-coated drug plates to test for susceptibility to varying anti-cancer therapies. Eight new cell lines were developed from various equine melanoma and tumor adjacent tissue. The behavior and morphology of established lines differ among patients and tumors. In the ongoing study, we will test the response of two selected lines to a panel of therapies using Biolog technology. 2D and 3D cell models were successfully developed from fresh and cryopreserved equine melanoma. This resource can be used to enhance our understanding of melanoma pathogenesis and therapeutic responses in further studies in human and veterinary oncology.

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Evaluating melanoma antigens (MAGE) in equine melanoma compared to healthy tissues

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As grey horses age, it is common for slowly spreading melanomas to develop at mucocuntaneous junctions. In contrast, colored horses may quickly develop melanomas that are fatally malignant. There are currently no effective treatments with melanomas reappearing very aggressively after surgical resection. To date, the molecular mechanism for malignancy is still unknown. However, over 35 years ago, the first tumor antigen genes, melanoma antigen genes (MAGEs), were discovered and targeted for immunotherapy in human melanoma patients. Our lab's previous research on the role of MAGEs in animal tissues suggested that they naturally protect male germ cells under stress, but this same molecular mechanism may also be used by cancer cells for self- preservation and therapy resistance. In this study, we investigated equine MAGE expression in both normal and melanoma tissues. Using >40 genes with MAGE homology in the equine genome, we designed equine-specific MAGE primers through NCBI's Primer-BLAST software. Trizol (guanidinium isothiocyanate–phenol) based RNA isolation was performed on >100 tissue samples, including melanoma, tumor-adjacent tissue and placenta. RNA was further purified using the OneStep PCR Inhibitor Removal Kit (Zymo) to reduce the contamination by melanin. Levels of gene expression were measured using reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) to determine their potential role in equine melanoma malignancy. Our preliminary data suggest that equine MAGEs also show tissue-specific expression profiles and are up-regulated in melanoma, which can be used for comparative research of the MAGE function, leading to novel therapy opportunities for humans and horses.

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Student Support: Texas Tech University SVM

Validation of a multi-purpose surgical model: “Popeye the Pug”

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Surgical skills and training are an integral part of veterinary medical education. Veterinary students face challenges in developing proficiency in surgical procedures due to limited training opportunities, cost, and time. This study's objective is to develop a surgical model that allows students to perform multiple small animal surgeries and conduct a validation study using veterinarians and students. The surgeries in this study include: proptosed eye replacement and tarsorrhaphy, third eyelid replacement using a pocket technique, meibomian gland adenoma removal and cosmetic repair, nasal wedge resection, and lip laceration and cosmetic repair. This study was conducted by videotaping participants performing the procedures and evaluating their ability to successfully complete the surgeries. The veterinarians completed a pre- and post- surgical evaluation survey, whereas the students completed only a post-surgical survey. This feedback helped further assess the realism and surgical training capabilities of the model.

Participants included twenty veterinary students in their second or third year and ten veterinarians. All figures and results were created using descriptive statistics. The results indicated that Popeye the Pug is a useful surgical model, with the ability to help students improve their instrument and suture handling while learning to perform multiple common small animal surgeries prior to attempting them in practice. Popeye has shown that the use of this model is useful to students and allows them to practice skills with low cost and low stakes.

Effects of Coastal Bermudagrass; fresh or hay, on equine fecal particle size, moisture content, and fiber content

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Impaction colic is a major cause of emergencies in equine practice. Dehydration, poor dentition, or low fiber quality may increase fecal particle size and decrease fecal moisture content, while increasing the risk for impaction colic in horses. The determination of how forage type (fresh or hay) affect fecal chemical and physical aspects may help define potential risk factors for colic in horses. The purpose of this experiment was to characterize the particle size, dry matter content, and fiberfractions of feces from healthy horses offered Coastal Bermudagrass forage, either fresh (pasture) or as hay . Twenty-seven horses (25 geldings and 2 mares; 14 Quarter Horses, 13 mixed breed) were used in a cross- over study design. Feces werecollected after 14 d of adaptation to diets. Spot fecal sampleswere dehydrated (55°C for 96 h) prior to analyses. Fecal dry matter content was adjusted by dehydrating subsamples at 100ºCfor 4 h. Fecal particle size was analyzed with a Gilson Fecal Particle Shaker using sieves of ?, ?, ?, ?, ?, and ? mm. Fiber fractions were determined by neutral (NDF) and acid detergent fiber (ADF) analyses. Data was found to be normally distributed and analyzed with a paired t-test (PRISM, GraphPad, Boston MA), with the fixed effects of ???? and the random effects of ???, while animal served as the experimental unit. Greater ($P < 0.01$) dry matter content was observed for horses consuming fresh pasture compared to hay (?? vs. ?? %). A greater ($P = 0.??$) percentage of fecal material was retained in the two smallest size sieves for horses consuming hay. Due to sample availability, the non-digestible component of feed was measured in only 20 horses. Greater ADF ($P < 0.01$) and NDF ($P < 0.01$) content was observed for horses consuming fresh pasture group compared to hay. Horse fecal particle size, dry matter content, and fiber fractions were affected by forage physical aspect. Horses consuming Coastal Bermudagrass fresh pasture had dryer, larger, and more fibrous feces compared to the same forage offered as hay. Further assessment is warranted to investigate the effects of fecal variables measured on colon motility.

Vaccine Efficacy of Non-Vaccinated Beef Cattle Fed Mycotoxin Blighted Feed

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Abstract: Mycotoxins are naturally occurring chemical compounds produced by fungi that are considered to affect the immune system and cause other health effects when ingested. Common mycotoxins include Aflatoxin, Fusarium, and tricothecens. The purpose of this study is to determine if aflatoxin blighted hay fed to naive beef cattle impacts the immune response of bovine respiratory complex vaccine by assessing virus neutralization (VN) of bovine herpesvirus type 1, bovine viral diarrhea types 1a/1b/2, parainfluenza 3 virus, and bovine respiratory syncytial virus. Bovine serology was pulled on days 0, 14, 21, and 30, ran through a centrifuge, and submitted to Texas A&M Veterinary Diagnostic Lab (n=5 per group). Cattle were randomly selected to group, vaccinated with a modified-live vaccine (MLV) against IBR, BVDV, PI3, and BRSV, and boosted on day 14. The treatment group was fed mycotoxin blighted hay (aflatoxin B-1 ~100 pbb) for two weeks and then pulled off. Control was fed mold-free grass hay. After analyzing the results from the study, the group that was fed aflatoxins had the seroconversion impacted vs. the control group. Day 30 serum levels of the treatment group could be indicative of a later seroconversion post removal of the aflatoxin Blighted hay. Results from this pilot study can further help the understanding of effects of mycotoxin hay on the bovine immune response post-vaccination.

NOTES: