Tuskegee Veterinary Scholars Program

Faculty Research and

2023 - 2024

Student Abstracts





Program Description

The Tuskegee Veterinary Scholars Program (TVSP) provides opportunities for pre-clinical veterinary students to be involved in mentored research over the summer period. The program

is conducted annually for 12 weeks from May. The purpose is to expose veterinary students to research and introduce them to research-oriented career options through



various activities. Each student will work on a project



with a Faculty Mentor. Students present their work at a symposium. Social activities and day trip/s.

Students recruited to T35 mechanism have an

additional 2-weeks, as the T35 program was designed for 12 weeks.

TVSP provides summer research experience through three (3) funded sub-programs: HRSA/HHS COE, Boehringer Ingelheim veterinary student scholars, and NIH T35 collaborative summer research experience with Mississippi State University College of Veterinary Medicine.

Research Faculty Interests

Woubit Abebe, DVM, MS, PhD

Professor and Director, Center for Food Animal Health Food Safety and Defense, Department of Pathobiology

Our lab, the Center for Food Animal Health, Food Safety and Defense, engaged in research related to food safety using cutting-edge technologies. Also, a multi-array platform has been developed and licensed to detect four priority foodborne pathogens that cause illnesses, outbreaks, and deaths yearly. In the past, we have engaged multiple DVM students in research on the dynamics of pathogen populations and quantification from different food sources. If interested, you can validate and quantify multiple salmonella serovars from various food sources. mainly chicken meat products. You can do molecular detection with real-time PCR quantification, sequencing, and pathogen characterization using bioinformatics, MALDI-TOF. The lab also has multiple PhD students willing to assist in your research endeavor daily.

Athema Etzioni, DVM, MS, DACVP

Chief, Clinical Pathology, Department of Pathobiology

- Clinical research projects, whether small, large or mixed. Research project may include working with our clinicians on clinical cases that present to TUCVM or research with one of our clinicians off site. Project may include collections of samples. Bloodwork trends in large and small animal patients that present to TUCVM with diarrhea.
- Poikilocytosis associated with diseased patients presenting to the TUCVM.

- Diagnostic sensitivity of direct smears vs fecal flotation to evaluate intestinal parasitic infections.
- Dental disease in small animals presenting to TUCVM.

Rawah Faraj, DVM, MS, PhD

Assistant Professor, Department of Pathobiology

Molecular detection of tick-borne disease from tick samples by PCR (Polymerase Chain Reaction) is a specialized facility focused on detecting and identifying pathogens transmitted by ticks using molecular techniques. These diseases may include Lyme disease, anaplasmosis, babesiosis, Rocky Mountain Spotted Fever and ehrlichiosis, among others. The laboratory utilizes PCR technology to amplify specific genetic material from the pathogens, enabling accurate identification, even when the pathogens are present in very low quantities.

Early detection of Leptospirosis from water samples using PCR (Polymerase Chain Reaction) is a crucial method for identifying the pathogen Leptospira bacteria, which can cause a wide range of symptoms in humans and animals. Leptospirosis is typically transmitted through contact with water or soil contaminated by the urine of infected animals, particularly rodents. Detecting the bacteria early, especially in environmental water sources, is vital for controlling outbreaks and preventing further spread.

Isolation and identification of bacteria causing dermatitis from water Samples. Dermatitis caused by waterborne bacteria is a significant health concern, especially in recreational water sources like swimming pools, lakes, and rivers. Bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus, and Aeromonas hydrophila are commonly associated with waterborne dermatitis. The process of isolating and identifying these pathogens from

water samples involves several steps, from sample collection to bacterial identification using microbiological, biochemical tests and PCR.

Dilip Gunturu, MS, PhD

Assistant Professor, Department of Biomedical Sciences

Pioneering Precision Medicine: Join a cutting-edge research team dedicated to revolutionizing cancer treatment through precision medicine. Explore how nanoparticles can be engineered to deliver drugs directly to cancer cells, minimizing side effects and maximizing therapeutic efficacy.

Cytokine Mapping in Cancer Therapy: Dive into the dynamic world of cytokines, the signaling proteins that dictate immune responses. Your work will directly contribute to understanding how these proteins influence cancer progression and therapy outcomes, paving the way for tailored treatment strategies.

Hands-On Experience with Advanced Technologies: Gain hands-on experience with the latest technologies in nanomedicine and cancer biology. Learn to manipulate nanoparticle carriers and assess their impact on cellular and molecular pathways in real-time.

Interdisciplinary Collaboration: Work alongside experts in pharmacology, oncology, and material science in a collaborative environment. This project offers a unique opportunity to bridge veterinary science and human medicine, highlighting the integral role of veterinary perspectives in advancing human health.

Contribute Publications: Participate in research that leads to high-impact publications. This is your chance to contribute to significant advancements in the field and establish a foundation for a possible career in veterinary research and beyond.

Mentorship and Career Development: Receive mentorship from leaders in the field and engage in discussions that could shape your future career. This project not only broadens your scientific knowledge but also expands your professional network in the biomedical sciences.

Abdelrahman Mohamed, DVM, MS, PhD

Associate Professor, Department of Pathobiology

- Molecular Characterization of environmental Enterococci and E. coli bacteria Isolated from Alabama Fish farm using Next-Generation Whole-Genome Sequencer.
- Molecular characterization of wild Aeromonas hydrophila using a Next-Generation sequencer.
- Identifying the Circulating Antibiotic Resistance Genes in Alabama Natural Waters.

Pawan Puri, DVM, PhD

Associate Professor, Department of Biomedical Sciences

Study how cells talk to each other to get their jobs done. We are especially interested in how this communication helps organs grow, how similar processes help organs repair themselves, and what goes wrong when these processes are out of control, leading to diseases.

We focus on a signaling enzyme called Protein Kinase A (PKA) and its role in kidney and stomach development. We also study how problems with PKA signaling can lead to cystic kidney disease and stomach cancer. To understand these issues, we use special mouse models, lab-grown

tissues, and cell cultures to mimic human diseases and uncover the root causes.

Students in the lab will have the chance to learn hands-on techniques like staining tissues to study proteins (immunohistochemistry), identifying proteins in samples (immunoblotting), amplifying DNA (PCR), and working with mouse models to better understand human diseases.

Sherein Salem, BVSc, MVSc, PhD

Associate Professor, Department of Pathobiology

Have you ever wondered what a diseased lung really looks like beyond X-rays and clinical signs? In our research project, we will dive into the world of veterinary forensic pathology by uncovering the hidden stories inside animal lungs through necropsy and histopathology.

Respiratory diseases are among the most common health issues in animals, often going undiagnosed until postmortem examination. By working hands-on with real necropsy cases, students will identify and analyze lung lesions, learning to distinguish between different types of pneumonia, hemorrhage, and other respiratory pathologies. This experience will provide a unique opportunity to connect clinical signs with pathological findings, helping students understand disease progression in a way that textbooks cannot.

Skills Students Will Gain:

- Necropsy Techniques Learn how to properly examine and collect lung samples from different species.
- Gross Pathology Interpretation Develop the ability to recognize key lung lesions and their significance.
- Microscopic Analysis Use histopathology to differentiate between various types of lung diseases.

- Diagnostic Reasoning Correlate clinical history with postmortem findings to propose potential causes.
- Research & Communication Skills Document findings, discuss cases, and present results like a pathologist-in-training.

By the end of our project, students will not only gain invaluable hands-on experience but also develop critical-thinking skills essential for careers in pathology, diagnostics, and research.

Teshome Yehualaeshet, DVM, PhD

Professor, Department of Pathobiology

Involved in teaching and research. Research focus is mainly: i) to develop improved quantitative and qualitative microbial diagnostic tools, ii) food safety and characterization of foodborne pathogens, iii) antibiotics resistance in foodborne pathogens, and iv) application of metabolomics in food safety. The incoming summer research student will involve in the application of metabolomics in food safety research.

2023 Student Abstracts

Hookworms and Roundworms in Dogs: A Retrospective Study of Comorbidities, Awareness, and Prevention

Briana Arceneaux and Athema Etzioni

Department of Pathobiology College of Veterinary Medicine Tuskegee University, Tuskegee, AL

Hookworms and roundworms are two helminths commonly affecting many dogs around the world. These intestinal worms can cause inflammation in the dog's gastrointestinal system and hookworms can cause lifethreatening anemia. When co-infection occurs with either of the helminths or other parasites, treatment may need to be more aggressive, and recovery may take longer. Performing diagnostic testing is important to assure the proper diagnosis is elucidated and the appropriate treatment is administered. The age range of dogs infected can vary, but helminth infections are more commonly seen in puppies. Over the years, pet owners have minimized the importance of intestinal worms, and many are not aware of methods of prevention or that zoonoses may occur. This study analyses current knowledge of ascarids and ancylostomatids, focusing on the awareness, importance of diagnostics, control methods and to prevent infection by good owner education and compliance.

Research Grant: TUCVM Veterinary Scholars Program &

Mississippi State CVM Student Support: NIH T35

Qualitative and Quantitative Assay of Salmonella Serovars from Poultry Products using Genovar and Nano Biosensors

<u>Kierney Burks</u>¹; Rawah Faraj¹; Rejoice Nyarku¹, Kingsley Bentum¹, Alocilja Evangelyn²; Woubit Abebe¹

¹Center for Food Animal Health, Food Safety, and Food Defense, Department of Pathobiology, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL; ²Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, Michigan

Outbreaks by Salmonella have not declined in the past fifteen years, and even worse, different Salmonella serotypes have emerged every year as the causative agents of foodborne illnesses. This study investigated the use of Magnetic Nanoparticles (MNPs) and Gold Nanoparticles (GNPs) to aid in the early detection of Salmonella in retail poultry products. Genovar microplate PCR assay was also used to qualitatively and quantitively determine the level of Salmonella serovars. Out of a total of 24 poultry products tested, 12 highly contaminated samples comprising chicken necks (5), turkey necks (5), and ground turkeys (2) were selected for further analysis. Briefly, 10gm of each sample was enriched in Tryptic Soy Broth, incubated overnight at 37°C, and then serially diluted 10⁻¹-10⁻⁸ for MNPs capture and direct plating (DP) of total Salmonella count.

Similarly, total DNA was extracted from the RV broth of each sample for quantitative and qualitative analysis using Genovar assay. Results showed that MNPs efficiently captured *Salmonella* when the level of contaminating *Salmonella* was higher than the overall

bacteria contamination. This is due to the nonspecific binding of MNPs to any bacteria in the sample. Observed by 18/13, 5/0, 20/45, 1/0, MNP CFU versus DP CFUs, respectively. However, GNPs can specifically detect Salmonella's presence in the abundance of other organisms. The abundance of Salmonella contamination with DP was recorded between 2X10⁷- 4.24X10⁹ per 10 gm of the sample. In all samples analyzed, chicken neck carried the most variable serovars, about 13 different salmonella serovars, followed by turkey neck with seven different serovars. Overall, the Genovar analysis revealed that Montevideo. Infantis. serovars Typhimurium, Saintpaul, and Hadar are the most abundant serovar from high to low ranging 3.5 X10⁵-2.36 GE/ul. A combination of MNP and GNPs will aid in the early detection of Salmonella. Nano-biosensors and an assay like Genovar will cut the time needed to know the serovar involved in the outbreak.

Research Support: USDA/NIFA/CBG 2021-38821-34710, MSU/USDA/NIFA RC113747TU, Student Support: DHHS/HRSA D34HP00001-35-00, NIH/NIMHD RCMI grant # U54MD007585

Identification of the Gastric Mesenchymal Cell Types Derived from Six2 Expressing Embryonic Progenitors by Lineage Tracing

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Mesenchymal epithelial crosstalk (MEC) is indispensable for gastric homeostasis. Mis-regulation of the MEC can cause gastric pathologies such as polyposis and cancer. Although mesenchyme is known to be a key player in gastric pathologies, the origin and identity of different mesenchymal cell types remain unknown. We recently characterized a novel conditional mutant Six2Cre^{+/-}-PKAcαR^{fl/wt} (CA-PKA) model in which single allele-mediated expression of constitutively active (CA) PKA (PKAcαR) was induced in the gastric mesenchyme using Six2-Cre transgenic mice. CA-PKA mice showed multiple gastric preneoplastic lesions such as marked inflammation, oxyntic atrophy, metaplasia, and invasive glands. The goal of the current study was to identify gastric mesenchymal cells derived from progenitors that may contribute towards the preneoplastic lesions in CA-PKA mice. We hypothesized that Six2 progenitors generate a subset of mesenchymal cells that are altered in CA-PKA mice. Lineage tracing in Six2-Cre;CAG-tdTomato mice along with immunofluorescence (Co-IF) were used to identify Six2progentior-dervied cells. We found that Six2 progenitorderived cells include ACTA2+ve cells of lamina propria, muscularis mucosae, muscularis externa as well as vascular smooth muscle cells. Gastric endothelial cells (CD31^{+ve}) are not Six2-progenitor-dervied.

progenitor-derived cells include a small subset of FSP1^{+ve} stromal fibroblasts. Further Co-IF analysis showed that all these cell types are markedly altered in Six2Cre^{+/-};CAG-tdtomato;PKAcαR^{fl/wt} mice. Our results identify Six2 progenitor-derived cells in the gastric mesenchyme that may be directly or indirectly involved in causing preneoplasia in CA-PKA mice.

Research Grant: This research was supported by grants from NIH T35OD010432, NIGMS# 1SC2GM130475 DHHS/HRSA D34HP00001-35-00, and NIH/NIMHD RCMI grant # U54MD007585

Student Support: National Institutes of Health project # 5T35OD010432

Effect Of Maternal Staphylococcus Aureus Mastitis Immunomodulatory Treatments on The Health Of Nursing Mouse Pups

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Mastitis is a disease that affects the mammary gland of lactating mothers. Mothers with infectious mastitis are recommended to breastfeed while on antibiotic treatment. However, exposure to antibiotics can affect both the mother and the child, therefore the need for alternate treatment is necessary. IgY, an antibody naturally derived from egg yolk is reported as a safer alternative to antibiotics because of its efficacy, specificity, and safety for oral consumption. One of the indicators of infant health is normal gastrointestinal (G.I.) motility. GI

motility can be compromised with poor treatment outcomes. The objectives of this study are to: (1) Compare the health outcome (growth and weight) of pups nursed from Staphylococcus aureus infected dams and treated with anti-SpA IgY, anti-SpA IgY+Vitamin D3, or anti-SpA IgY+Vitamin D3+RP. (2) Evaluate the degree of gastrointestinal motility of pups from Staphylococcus aureus infected dams and treated with each therapy. Fifty pups (five males and five females per treatment) were collected. The litter size and growth rate of these were determined during treatment until they were weaned. Colon samples were collected from each for Angiotensin II receptor analysis via qPCR.

Research Grant: TUCVM Veterinary Scholars Program; Student Support: Boehringer Ingelheim and HHS-COE

Vitrification of Different Volumes of Canine Epididymal Spermatozoa in Semen Straws: Effects on Cryosurvival

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Vitrification, a method of cryopreservation, has advantages over conventional slow freezing including the reduction of intracellular ice crystallization. In our previous study (Mason et al, NVSS 2022), the cryosurvival of canine spermatozoa was better when vitrification pellet size was larger (~10 μl vs ~20 μl). The present study examined the effects of packaging different

volumes in semen straws on the post-thaw cryosurvival. Sperm samples were extracted from cauda epididymis of dogs using PBS, centrifuged, and transferred to an extender containing 20% egg yolk. In Experiment 1, we tested 4 treatments: 20 µl, 60 µl, 100 µl, and 140 µl. In Experiment 2, because of improved sperm parameters in the 60µl of experiment 1, more refined volumes of 30 µl, 40 μl, 50 μl, 6 0 μl, and 70 μl were evaluated. Resuspend samples were cooled to 4 degrees for 2 hours; specific volumes were loaded into 0.25 ml straws which were then sealed and placed in 0.5 ml straws. The samples were vitrified by directly plunging the straws in liquid nitrogen. We tested eight testes per experiment. Post-thaw total motility (TM) and total progressive motility (TPM) were evaluated by computer-assisted sperm analysis and data analyzed using one-way ANOVA. In experiment 1, the highest post-thaw motility was observed in the 60ul with a moderate treatment effect (P=0.07). In experiment 2, volume had little effect on the post-thaw parameters (P>0.7). Our observations indicate that vitrification of spermatozoa in 0.25 ml straws in volumes greater than 30 μl supports better post-thaw motility than vitrification in smaller pellets. This counteracts the dogma that vitrification of cells in the smallest volumes supports better cryosurvival.

Research Support: NIH T35OD010432 Student Support: HRSA D34HP00001-35-00

Irinotecan Loaded Chitosan-Nanoparticles: Synthesis, Characterization and Invitro Evaluation against Colorectal Cancer

Amol Morrow, Dilip Reddy Gunturu and Temesgen Samuel

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We investigated the effect of irinotecan, a topoisomerase-I inhibitor, on colorectal cancer cells using chitosan nanoparticles. Colorectal cancer is a common and deadly disease that requires effective treatment. Topoisomerase inhibitors are drugs that induce cell death by interfering with DNA replication. However, they have serious side effects and limited efficacy. Nanoparticle drug delivery systems can enhance the performance of anticancer drugs by improving their bioavailability, biodegradability, and safety. We prepared irinotecan-loaded nanoparticles using chitosan-sodium tripolyphosphate and chitosanpolyethylene glycol-sodium tripolyphosphate. Chitosan biocompatible muco-adhesive polymer. characterized the nanoparticles for their size, shape, and loading efficiency. We also evaluated their cytotoxicity and cell cycle arrest on colorectal cancer cells. Our results that chitosan encapsulated nanoparticles reduced cell viability more effectively than standard irinotecan. Our study suggests that chitosan nanoparticles can be a promising carrier for irinotecan delivery in colorectal cancer treatment.

This research was supported by grants from NIH T35OD010432, DHHS/HRSA D34HP00001-35-00, and NIH/NIMHD RCMI grant # U54MD007585

Molecular Characterization of *Listeria*Monocytogenes Isolated from the Cow-Calf Farming System of Central Alabama

Sabria Wooten, Woubit Abebe, Rawah Faraj

Center for Food Animal Health, Food Safety, and Food Defense, Department of Pathobiology, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL

Listeria monocytogenes is a ubiquitous microorganism and the causative agent of Listeriosis. L. monocytogenes can be found in moist environments, soil, water, and animals. This study analyzed (50) fecal samples collected from cow-calf farming systems between May 2023 and July 2023 from different farms in central AL, USA. We examined the prevalence, virulence factors, and phylogenetic distribution of the *hlyA* gene of L. monocytogenes isolated from the cow-calf farming The prevalence of contamination system. L. monocytogenes was 11 (22%) out of 50 fecal samples. Serotypes and virulence genes of all L. monocytogenes positive samples were characterized using multiplex PCR. All isolates were positive for inlA, inlB, plcB, prfA, iap, actA, hly, hlyA, and Lmo0733 genes. PCR-based serotyping showed the isolates belonged to serotype 4d (4/11, 36%), 1/2b (2/11, 18 %), 4a (3/11, 27%), and 1/2a (2/11, 18%). The presence of variations of the *hlyA* genes among the isolates was analyzed using geneious prime software. The results of hlvA gene alignment from all isolates revealed high similarity with strains reported from different parts of the world (GenBank Database). This gene will be used to understand the evolutionary distance of the current L. monocytogenes isolates with respect to those available in public domains. The high

percentage of *L. monocytogenes* isolation in this study may reflect an increased risk of infection through direct or indirect contact with cow fecal samples. Regular screening of animals for *L. monocytogenes* is beneficial for preventing listeriosis in farms and, subsequently, the food system.

Research Support: USDA/NIFA/CBG 2021-38821-34710, MSU/USDA/NIFA RC113747TU, Student Support: DHHS/HRSA D34HP00001-35-00, NIH/NIMHD RCMI grant # U54MD007585





2024 Student Abstracts

Prevalence and Molecular Detection of Rocky Mountain Spotted Fever in Ticks from Dogs and Pigs in the Eastern Area of Alabama

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Department of Pathobiology, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL

Rocky Mountain Spotted Fever (RMSF) is a lifethreatening tick-borne disease caused by the gramnegative, intracellular bacteria Rickettsia rickettsii. that infects vascular endothelium cells. RMSF is the most common rickettsial disease in the United States and mostly affects the Southeast. This study analyzed 50 tick samples collected from dogs and pigs between May 2023 - July 2024 from different counties in the east-central area of AL. Individual ticks were processed for DNA extraction and molecular detection of Rickettsia spp. using the PCR method. The outer membrane protein A (ompA), outer membrane protein B (ompB), and hightemperature Requirement A (htrA) gene were used as genetic markers. All rickettsial genes were positive in ticks from dogs and pigs. The prevalence of incidence of Rickettsial spp. including RMSF was 15 out of 50 (30%) tick samples from the animals. Most of the sequenced PCR products were homologous to R. rickettsii and Rickettsia spp. We analyzed the DNA sequences using the NCBI Blast software program and found 94% similarity between our samples and other Rickettsia rickettsii spp. Additionally, our samples displayed 93% similarity with other Rickettsial spp. in the Spotted Fever Group. Infected dogs serve as sentinels to indicate the presence of infected ticks in the east-central area of AL.

Macon, Lee, Bullock, and Montgomery County residents are at considerable risk of exposure to the SFG including RMSF. With this exposure risk, we can form a brief curriculum that covers preventative measures, signs for both humans and animals, and a plan of action for each stage of infection. Making this knowledge common among residents will help decrease the number of tick bites and the severity of infections as we catch them in earlier stages and begin the proper treatment.

Research Support: NIH/NIMHD RCMI grant # U54MD007585 and NIH T35OD010432 Student Support: DHHS/HRSA D34HP00001-35-00 and NIH T35OD010432

Extracellular Vesicles (EVs) and Canine Sperm Motility: Investigating the Role of Seminal Fluid EVs

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Extracellular vesicles (EVs) have emerged as essential carriers for transporting proteins, lipids, and nucleic acids throughout the body. These vesicles, including exosomes, microvesicles, and apoptotic bodies, play diverse roles in cell-to-cell communication. While their significance has been well-studied in porcine and bovine species reproduction, the effects of EVs on sperm motility in canines remain to be fully understood. Objective: This study aims to investigate the hypothesis that EVs isolated from male seminal fluid can modulate canine sperm motility. By analyzing sperm motility parameters and examining the uptake of fluorescently

labeled EVs, we explore the direct effects of these vesicles on canine sperm function. Methods: Seminal fluid EVs were extracted from the epididymis tail using size exclusion and centrifugation techniques. Sperm were isolated from the less active compartment, the epididymis corpus, through low-speed centrifugation. Sperm were incubated with seminal fluid EVs for varying durations (0, 1, and 2 hours). Computer-assisted sperm analysis was used to assess sperm motility parameters. EVs containing proteins and mRNA were fluorescently labeled. The uptake of labeled EVs by sperm was examined. Results: After 1 hour of incubation, sperm demonstrated uptake of labeled EVs. A consistent trend of increased sperm motility percent was observed with increased doses of EVs. Statistically significant improvements in sperm motility were seen after 1 hour of incubation at the highest EV dose. Other sperm motility parameters, including straight-line velocity, linearity, and straightness, also increased with escalating EV doses. Conclusion: These findings suggest that EVs from male seminal fluid directly influence canine sperm motility. Further research in this area could enhance our understanding of reproductive mechanisms and potentially lead to novel therapeutic approaches for improving fertility in canines.

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Characterization of Immune Cell Markers and Inflammation-Related Signaling Pathways in a Novel Mouse Model of Gastric Preneoplasia

Brooke Keys, Esraa Alnahrawy, Fentahun Abate-and Pawan Puri

Department of Biomedical Sciences, Tuskegee University, College of Veterinary Medicine, Tuskegee, AL

Gastric cancer is the fourth leading cause of cancer deaths worldwide. Inflammation is a key contributor to gastric carcinogenesis. We recently characterized Six2Cre+/-PKAcaR^{fl/wt} (CA-PKA) mouse model in constitutively active (CA) Protein Kinase A (PKAcaR) was expressed in the gastric mesenchyme using Six2-Cre transgenic mice. CA-PKA stomach showed marked infiltration of inflammation cells, upregulation of inflammatory mediators and preneoplastic lesions. However, the expression of immune cell markers and the status of inflammation-associated pathways in CA-PKA mice remain unclear. We hypothesized that the infiltrating inflammatory cells in the developing stomach of CA-PKA mice express distinct immune markers and show activation of inflammation-related signaling. Histology and immunofluorescence (IF) with leukocyte marker CD45 confirmed inflammatory cell infiltration. We found that a very small proportion of CD45^{+ve} cells expressed T cell marker CD3. Further IF analyses showed many leukocytes expressed arginase 1, a myeloid-derived suppressor cell marker. We analyzed NFKB1 and phospho (p)-CREB, well established regulators of inflammatory pathways. NFKB1 signal was cytoplasmic in the gastric epithelial cells of both control and mutant stomach as well as in the infiltrating CD45^{+ve} leukocytes

in mutants. Co-IF with (p)-CREB and CD45 antibodies in the control stomach showed that most of the resident CD45^{+ve} immune cells were already p-CREB^{+ve}; in the mutant stomach, p-CREB^{+ve}/CD45^{+ve} cells were markedly upregulated, although no apparent change in p-CREB intensity was observed. Together our results reveal immune markers and status of inflammation-related signaling pathways in a novel model of gastric preneoplasia.

Research Grant: This research was supported by grants from NIH T35OD010432, NIGMS# 5R16GM149389 DHHS/HRSA D34HP00001-35-00, and NIH/NIMHD RCMI grant # U54MD007585

Student Support: National Institutes of Health project # NIH T35OD010432

Investigation of Antimicrobial Resistance and Virulence of Escherichia coli and Klebsiella sp. in Pet Reptiles

<u>Jean Linn</u>, Yesut<mark>or Soku, Abdel</mark>rahman Mohamed, Steven Walker, Athema Etzioni

Pathobiology and Clinical Sciences Departments, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL

Pet reptiles can become reservoirs for potential zoonotic bacteria, including some *Escherichia coli* and *Klebsiella* species. This study aims to investigate the antimicrobial resistance (AMR) profile of *E. coli* and *Klebsiella* in pet reptiles and characterize the virulence factors present in these bacteria. In this study, cloacal swabs from 18 pet reptiles, including ball python, cottonmouth vs.

copperhead hybrid, copperhead, Nile monitor, green iguana, and savannah monitor, were sampled. Samples were cultured in tryptic soy broth (TSB), chrome select agar, MacConkey agar, and Levine EMB agar. Isolates that were pink on MacConkey, blue on chrome select agar, and green metallic sheen with a darker center on EMB agar were further purified and identified using a Biolog Gen III machine. Three E. coli, 3 Klebsiella oxytoca, and 1 Klebsiella pneumoniae were isolated from the samples. Antimicrobial susceptibility testing (AST) was conducted on E. coli and Klebsiella spp. using ampicillin. gentamicin. cefepime, trimethoprimsulfamethoxazole, tetracycline, and azithromycin, following CLSI guidelines. Zones of inhibition were interpreted based on CLSI criteria. E. coli was resistant only to ampicillin. Klebsiella spp. was found to be resistant to ampicillin, cefepime, and tetracycline. PCR was conducted with the *E.coli* samples and showed no virulence factors except one sample positive for the uidA gene. Sequencing and virulence characterization for all isolates are pending. Using a diverse sampling of pet reptiles, we aimed to reveal enteric bacteria that house virulence factors and antimicrobial resistance, posing a potential risk to their caretakers and, on a broader scope, to public health.

Research Grant: NIH/NIMHD RCMI grant # U54MD007585, DHHS/HRSA grant # D34HP00001-35-00, and grant # NIH T35OD010432 Student Support: DHHS/HRSA grant # D34HP00001-35-00 and T35 student grant # NIH T35OD010432

The Cytokine Response of Irinotecan-Loaded Nanoparticles Unmasked: Developments in Colorectal Cancer Treatment

Jennifer Maharath, Dilip Reddy Gunturu and Temesgen Samuel

Department of Biomedical Sciences (Maharath, Gunturu) and Pathobiology (Samuel), College of Veterinary Medicine, Tuskegee University, AL

Colorectal cancer (CRC) is the third most prevalent cancer affecting both men and women worldwide. Despite being a standard method of treatment. conventional chemotherapy significant presents challenges due to its adverse effects and suboptimal pharmacological properties. Consequently, there is an urgent need to explore alternative therapeutic approaches that focus on the efficacy and safety of CRC treatment. Recent studies have demonstrated the success of nanoparticle formulations in drug delivery, significantly enhancing pharmacological properties and minimizing its harmful effects. Additionally, therapy-induced cytokine response is gaining traction as a crucial aspect of precision medicine. This study aims to evaluate the efficacy of irinotecan, a topoisomerase-I inhibitor, in comparison to irinotecan-loaded nanoparticles. IL-22 has been shown to play a significant role in several regenerative processes while supporting tumorigenesis. Due to the limited data and prognostic significance of IL-22's role in CRC, it has become an important target for therapeutic development. examining the effects of irinotecan-loaded nanoparticles on cytokine signaling and cell viability, we aim to improve the therapeutic potential of this approach, paving the way for future advancements in precisionbased treatment protocols for CRC.

Research Grant: DHHS/HRSA D34HP00001-35-00 and

NIH/NIMHD RCMI grant # U54MD007585

Student Support: NIH T35OD010432 and Boehringer

Ingelheim

Investigating the Effect of *Aeromonas hydrophila* **Infection on Serum Chemistry of Channel Catfish**

<u>Savannah McCrobie</u>, Yesutor Soku, Miles D. Lange, Abdelrahman Mohamed

Department of Pathobiology, Tuskegee University CVM, Tuskegee, AL (McCrobie, Soku, Mohamed) United States Department of Agriculture, Agriculture Research Service, Aquatic Animal Health Research Unit, Auburn, AL (Lange)

Aeromonas hydrophila (A. hydrophila) is a hemorrhagic and septicemic pathogen that causes fatal disease in channel catfish (*Ictalurus punctatus*). Infection has been shown to increase in severity when exposed fish have a compromised skin barrier as well as after feeding. While the exact pathogenesis is unknown, concentration of blood flow in the gastrointestinal tract after eating is suspected to exacerbate the disease. To simulate a break in the skin barrier, some of the fish received a clip on the fin. The catfish were separated into two control groups and four experimental groups. The control groups were fin clipped and fed (FCF) and not fin clipped and fed (NCF). The experimental groups were FCF, NCF, fin clipped and not fed (FCN), and not fin clipped and not fed (NCN). Each group consisted of one hundred fish which were infected with the ALG-15-097 strain of A. hydrophila and then had their blood collected at two, four, and eight hours post infection. Any convalescing fish

also had a final sample drawn from them. Serum was collected from all the blood samples and processed using a Vetscan VS2 Chemical Analyzer. The serum chemistry values obtained will be analyzed to determine the effects of infection on the chemistry profile, which will allow greater insight into the pathology and which systems are most affected by the disease.

Research Support: NIH/NIMHD RCMI grant #

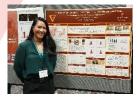
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