Tuskegee Veterinary Scholars Program

Faculty Research Interests

and

2024 – 2025

Student Research Abstracts

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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.

TVSP provides summer research experience through three (2) funded sub-programs: HRSA/HHS COE, and Boehringer Ingelheim Veterinary Scholars Program (BIVSP).

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, bioinformatics, and pathogen characterization using MALDI-TOF. The lab also has multiple PhD students willing to assist in your research endeavor daily.

Benjamin Adu-Addai, DVM, PhD
Associate Professor, Department of Biomedical Sciences

My research focuses on addressing emerging and re-emerging diseases through improved treatment and prevention strategies. It involves identifying and disrupting key signaling mechanisms involved in disease progression, as well as enhancing targeted drug-delivery approaches. My current work evaluates the combined effects of IgY, cholecalciferol, and the novel peptide RP185 on *Staphylococcus aureus*–induced mastitis and osteomyelitis. This study targets the major bacterial evasion mechanisms, including internalization by mammary epithelial cells, intracellular replication and biofilm formation, evasion of phagocytosis, resistance to

macrophage-mediated killing, destruction of phagocytes, and antibiotic resistance.

Noriko Aoi, BVSc, MVSc
Clinician, Dentistry and Oral Surgery Service,
Veterinary Teaching Hospital
Assistant Professor, Department of Clinical Sciences

Small animal dentistry has advanced tremendously over the past few decades, making strong clinical skills and evidence-based knowledge essential for current veterinary practice. Through the Tuskegee Veterinary Scholars Program, students gain experience in data collection and analysis and conduct literature reviews. Students will also observe and participate in clinical cases, assist with procedures, and develop diagnostic and technical skills in small animal dentistry.

Prior clinical experience is not required. Research students are expected to demonstrate strong work ethics, including reliability, diligence, and the ability to work independently when given guidance.

Previous research topics include:

- Anatomical Characteristics of the Roots of Maxillary First Molar Teeth in Dogs Assessed by Computed Tomography (CT) *student oral presentation award
- Correlations Between Periodontal Disease and Systemic Health using Dental Radiographs and Clinical Pathology
- Comparison of Prevalence of Intestinal Helminths in Dogs and Cats against the History of Anthelmintic and Preventive Products Usage
- Prevalence of Intestinal Helminths in Shelter Dogs *student oral presentation award
- Effects of Heat Pretreatment Method on Heartworm Antigen Detection on Dogs *student oral presentation award

- Complete Blood Cell Counts and Serum Chemistry of Dogs Undergoing Dental Procedures: A Comparative Study
- Comparison of Fecal Flotation and Centrifugation Techniques for the Diagnosis of the Most Common Intestinal Nematode Parasites in Dogs *student poster presentation award
- Effectiveness of Training in Communication, Skills for Fourth-Year Veterinary Medical Students in TUSVM Small Animal Outpatient Rotation

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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, BVSC&AH, MVSc, 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 **liver disease** truly looks like beyond serum chemistry values and imaging findings? In this research project, students will explore the field of veterinary forensic pathology by investigating hepatic diseases through systematic necropsy and histopathological examination.

The liver is a central organ involved in metabolism, detoxification, circulation, and immune regulation, making

it a key indicator of both primary and systemic disease. Hepatic disorders are common across animal species and frequently remain undiagnosed until postmortem evaluation. Through hands-on work with real necropsy cases, students will identify and characterize a wide spectrum of hepatic lesions, including inflammatory, degenerative, metabolic, vascular, and fibrotic changes. This experience will allow students to correlate clinical history and laboratory findings with gross and microscopic pathology, providing a comprehensive understanding of disease processes that cannot be achieved through textbooks alone.

Skills Students Will Gain:

- **Necropsy Techniques** – Learn proper examination and sampling of the liver from different animal species.
- **Gross Pathology Interpretation** – Develop the ability to recognize major hepatic lesion patterns and their diagnostic significance.
- **Microscopic Analysis** – Use histopathology to differentiate between various types of hepatic diseases.
- **Diagnostic Reasoning** – Correlate clinical history and postmortem findings to propose potential causes of disease.
- **Research & Communication Skills** – Document findings, discuss cases, and present results in a professional, pathology-focused format.

By the end of this project, students will gain valuable hands-on experience and develop critical-thinking skills essential for careers in veterinary pathology, diagnostics, and research.

Gemechu Wirtu, DVM, MS, PhD
Professor, Department of Biomedical Sciences

Research interest/areas

- Basic and applied aspects of mammalian gamete and embryo biology with the goal of improving the success of assisted reproductive technologies.
- In vitro fertilization and embryo culture
- Cryopreservation of gametes (spermatozoa and oocyte)
- Apiculture
- Lipid metabolism

Students joining my lab will gain hands-on experience in animal reproductive biology and applied research. Opportunities include working with canine and feline gonads (ovaries and testes) to recover and evaluate gametes (oocytes and spermatozoa) and to investigate factors influencing fertilization and early embryo development.

In addition, students will have the opportunity to participate in a mini research project focused on honeybee health and productivity, with field-based work in local apiaries.

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.

2024 Student Abstracts

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

Laila Barnes, Quintera Gordon, Rawah Faraj*
Department of Pathobiology, College of Veterinary Medicine, Tuskegee University, Tuskegee, AL

Rocky Mountain Spotted Fever (RMSF) is a life-threatening tick-borne disease caused by the gram-negative, 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 high-temperature 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

Loren Brown, Gemechu Wirtu, and Toufic Nashar
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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.

Acknowledgements: DHHS/HRSA D34HP00001-35-00 for student and research support, and NIH/NIMHD RCMI grant # U54MD007585 for research support, and NIH T35OD010432 for both research and student support

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

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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 which 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 $p\text{-}CREB^{+ve}$; in the mutant stomach, $p\text{-}CREB^{+ve}/CD45^{+ve}$ 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

Jean Linn, Yesutor Soku, Abdelrahman 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, trimethoprim-sulfamethoxazole, 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 presents significant 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. By 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 precision-based 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

Savannah McCrobie, Yesutor Soku, Miles D. Lange, Abdelrahman Mohamed
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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 # U54MD007585 and NIH T35OD010432
Student Support: DHHS/HRSA D34HP00001-35-00 and NIH T35OD01043


THIS IS WHAT A VETERINARY SCHOLAR LOOKS LIKE
MARS
VSS

Anatomical Characteristics of the Roots of Maxillary First Molar Teeth in Dogs Assessed by Computed Tomography

Amber D. Cooper, Marc Alkhal, and Noriko Aoi

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

Veterinary practitioners often face challenges in evaluating and treating the maxillary first molar teeth in dogs due to their caudal location within the oral cavity and the presence of adjacent structures such as the hard palate, zygoma, cheek tissue and orbit. These teeth have three roots—mesial, distal, and palatal—and are positioned adjacent to the fourth premolars and second molars, leading to overlapping structures on intraoral radiographs. This superimposition, combined with a limited understanding of root morphology, can complicate extractions, periodontal assessment, and other dental procedures. In this study, computed tomography (CT) was used to obtain detailed images of the maxillary first molars in 20 adult canine cadavers. Each of the three roots was measured for length and width at both the base and mid-root levels. Additionally, the presence and direction of root curvatures were evaluated. The mean lengths of the mesial, distal and palatal roots of the right and left maxillary first molars were 10.8 mm and 10.7 mm, 10.3 mm and 10.2 mm, 8.4 and 9.6 mm, respectively. The mesial and distal roots were similar in length, while the distal roots were approximately 20% shorter. Root widths were reduced at the mid-root by 29-37% compared to the base. Approximately 80-88% of the roots demonstrated curvature. These findings provide a more detailed anatomical understanding of this region and support more effective clinical management of maxillary molars in dogs.

Research and Student Support: Grant# DHHS/HRSA D34HP00001-35-00

Tracking Waterborne Pathogens: Isolation and Molecular Detection of Pathogenic *Escherichia Coli f*rom Lake Tuskegee in Tuskegee, Alabama

Quintera Gordon, Alexis Smith, Rawah Faraj*

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Escherichia coli is a waterborne pathogen that poses a significant public health risk, especially in recreational and surface water bodies. Although many *E. coli* strains are harmless commensals, pathogenic types can cause severe gastrointestinal illness and complications, including hemolytic uremic syndrome. This study aimed to evaluate the microbial quality of Lake Tuskegee in Alabama by detecting *E. coli* contamination and assessing the prevalence of the virulence-associated eye gene through molecular techniques. A total of 100 water samples were collected from different locations around the lake. Presumptive *E. coli* isolates were obtained using selective culture media, followed by DNA extraction and PCR amplification of the 16S rRNA gene for confirmation. Additionally, PCR was employed to detect the eaeA gene, which encodes the intimin protein linked to EPEC strains. Results showed that 94% of the samples tested positive for *E. coli* strains based on 16S rRNA gene amplification, and 43% of these isolates contained the eaeA gene, suggesting potential pathogenicity. Sequence analysis with the NCBI BLAST tool revealed 100% similarity between our isolates and reference sequences of *E. coli* strains, including *E. coli* O157:H7. The detection of strains expressing the eaeA gene in Lake Tuskegee indicates a potential health threat to individuals exposed to the water. These findings highlight the importance of continuous microbial monitoring and the implementation of effective water quality management strategies to reduce the risk of waterborne disease transmission.

Research and Student Support: DHHS/HRSA D34HP00001-35-00, Boehringer Ingelheim Veterinary Scholars Program, and NIH/NIMHD RCMI grant # U54MD007585

Metabolomics Application: Authentication and Detection of Antibiotic Residues in Milk

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[1]College of Veterinary Medicine, [2]Department of Chemistry, Tuskegee University, [3]Department of Large Animal Clinical Sciences, Tuskegee University, [4]Auburn University Department of Science and Mathematics, Auburn University

Milk is a staple in the global food supply, making its authenticity and safety crucial for public health. This study explores the application of metabolomics in authenticating milk and detecting antibiotic residues which are important key aspects of food safety. We employed Nuclear Magnetic Resonance (NMR) spectroscopy to analyze the metabolomic profiles of various milk types, including cow, goat, and plant-based alternatives. Antibiotic residue detection was conducted using mass spectrometry (MS) analysis.

Milk samples were sourced from farms and retail stores to ensure diversity and representativeness of the samples. Each sample underwent a standard metabolomics to extract the metabolites. NMR samples were reconstituted with D_2O and analyzed using TopSpin and MetaboAnalyst 6.0. For the MS analysis, the control antibiotics standard used were erythromycin, sulfamethoxazole, penicillin, and tetracycline.

Our findings indicate that cow's milk has higher sugar and citrate content compared to goat milk, while oat milk among plant-based options contains the most lactose and glucose. ANOVA confirmed that pasteurized cow's milk had significantly higher lactose-to-sucrose ratios than unpasteurized and goat milk. This study has laid the groundwork for a validated protocol in metabolomic profiling and detection antibiotic residue, highlighting the potential of metabolomics in milk authentication and future applications in food safety regulation and quality control. Keywords: Mass Spectrometry, Nuclear Magnetic Resonance, Antibiotic Residues, & Milk Authentication.

Research Support: DHHS/HRSA D34HP00001-35-00; Boehringer Ingelheim Veterinary Scholars Program,

NIH/NIMHD RCMI grant # U54MD007585; NMR Grant: NSF MRI CHE 1427568

Precision Diagnostics: Serovar-Specific Marker Identification in *Glaesserella parasuis* Marker Identification via Pangenome Profiling

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Glaesserella parasuis (*G. parasuis*) is a commensal bacterium of the porcine upper respiratory tract and an opportunistic pathogen responsible for Glässer's disease, which primarily affects young pigs and causes major economic losses worldwide. The disease is characterized by polyserositis, polyarthritis, and pneumonia. Because of its fastidious growth requirements, traditional culture-based diagnostics are labor-intensive and slow, underscoring the need for rapid molecular detection methods. A comprehensive understanding of genomic diversity and virulence factors across the 15 recognized serovars is essential for developing novel diagnostics. In this study, we employed a pangenomic approach to analyze 46 *G. parasuis* genomes retrieved from NCBI. Genome quality was assessed using CheckM, and serotype classification identified serotypes 4, 5, and 7 as the most prevalent. Pangenome analysis with the Roary ILP pipeline revealed serovar-specific genes, while core genome analysis across identity thresholds identified 1,199 to 1,144 conserved genes at 65% to 95%, respectively, including 168 hypothetical proteins at the 95% threshold. Notably, key virulence- and fitness-associated genes were detected, including *arnC_2*, *cdtB_1*, and *mshA*. Certain hypothetical proteins were uniquely associated with serotypes 5 and 11, while *epsE_1*—a glycosyltransferase involved in exopolysaccharide biosynthesis—was specific to serotype 7.

The minimal cross-reactivity observed among these targets highlights their potential utility in serotype-specific molecular diagnostics. These findings provide a foundation for the development of precise molecular assays and offer novel insights into the role of previously uncharacterized proteins,

with implications for improved diagnostics, vaccine development, and disease control strategies in swine populations.

Research Support: DHHS/HRSA D34HP00001-35-00, Boehringer Ingelheim Veterinary Scholars Program, and NIH/NIMHD RCMI grant #U54MD007585. The study was also partly supported by a grant from USDA/NIFA/CBG 2021-38821-34710

The Use of Traditional Fecal Diagnostics Versus Fecal PCR to Detect Intestinal Parasites

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Trichostrongylus spp. are gastrointestinal nematodes that significantly threaten the health and productivity of ruminants, especially in organic beef cattle, where anthelmintic use is limited. This study aimed to compare the effectiveness and diagnostic accuracy of the traditional method (fecal egg count) with polymerase chain reaction (PCR) for detecting Trichostrongylus spp. in fecal samples from organic beef cattle. A total of 9 fecal samples were collected from cattle raised in certified organic systems. All samples were initially examined using direct smear, fecal float, egg per gram (McMaster), followed by molecular identification targeting the cytochrome oxidase subunit 1 (COX1) gene. Results showed that while traditional fecal egg counts detected Trichostrongylus ova in 3 of the samples, PCR analysis identified parasite DNA in 2 samples. The molecular method also enabled accurate species-level identification, which is not possible with conventional microscopy. These findings highlight the limitations of traditional diagnostic methods and emphasize the importance of incorporating molecular diagnostics like PCR to improve parasite monitoring and control in organic livestock systems.

Student and Research Support: DHHS/HRSA D34HP00001-35-00, Boehringer Ingelheim Veterinary Scholars Program, and NIH/NIMHD RCMI grant #U54MD007585

Characterization of Antimicrobial Resistance in Escherichia Coli Isolated from Central Alabama Freshwaters

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Freshwater systems in Alabama are vital resources for both recreation and agriculture, yet their microbial safety remains under-monitored. In this study, we characterized the antimicrobial resistance (AMR) profile of Escherichia coli from surface water samples collected from publicly accessible freshwater sources across six counties in Alabama, USA. We surveyed 50 freshwater sites across Autauga, Chambers, Elmore, Lee, Macon, and Montgomery counties in Alabama and isolated non-pathogenic E. coli from 12 locations (24%) using the Biolog GEN III Microplate platform. Phenotypic profiling by Kirby–Bauer disc diffusion revealed universal resistance to ampicillin (12/12), with 25% resistance to tetracycline (3/12), 25% to gentamicin (3/12), and 8% to sulfamethoxazole/trimethoprim (1/12). Among E. coli isolates, single-drug resistance predominated (8/12), double-drug resistance was observed in 2/12, and multidrug resistance (≥3 classes) in 2/12. Whole-genome sequencing identified β-lactamase genes in 6/12 isolates and resistance determinants for cephalosporins, aminoglycosides, and tetracyclines each in 5/12. Genotype–phenotype concordance was complete for ampicillin (6 genes positive isolates all resistant) but lower for gentamicin (1:3). Numerous virulence genes related to adhesion (fimA, faeC), biofilm formation (csgB, csgG), quorum sensing (lasl, pchB), iron acquisition (entA), secretion systems (gspC, gspD), and motility (flgB, fliG) were also detected. The presence of both genotypic and phenotypic resistance, along with virulence genes in E. coli from publicly accessible

freshwater sources, highlights the urgent need for systematic environmental surveillance of antimicrobial resistance.

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Factors Affecting Prevalence and Load of Varroa Mite in Apiaries in Macon County

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The ectoparasitic mite Varroa destructor, originally parasitizing the Asian honeybee (Apis cerana), has emerged as a global threat to apiculture following its host shift to the European honeybee (Apis mellifera). Reproduction typically occurs in drone brood cells, which are large and have longer capping stages, allowing more time for mite reproduction. Besides feeding on the larvae, pupae and adult bees, varroa mites transmit virus particles directly into the bees leading to immunosuppression and other disorders. Despite the implementation of numerous control strategies, the interplay between the mite and environmental or biological variables continues to jeopardize colony viability and apicultural productivity. The objective of the present study was to determine the prevalence and some factors affecting the parasitic load of varroa in Macon County, Alabama. Varroa infestation was assessed using the alcohol wash method during the months of May, June and July. Approximately 300 bees were sampled from brood nests, placed in 125 mL of 70% isopropyl alcohol, and shaken to dislodge mites for counting. A total of 19 hives were examined in three bee yards. The overall prevalence was 93.7% (15 of 16 hives examined), with a parasite load ranging from 0.003 to 0.03 mites per bee (0.3 to 3%). The proportion of hives with mite loads between 0 and 1% and between 1 and 3% was 33% and 67%, respectively. The results confirm a high prevalence of varroa mites in the county. There is a need for ongoing surveillance and evidence-based mite management strategies. There was a tendency for higher parasite load in hives with a large population of bees.

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Pathology Unveiled: Investigating Lung lesions through Gross and Histopathological Examinations

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From May 12 to July 12, 2025, a comprehensive postmortem investigation was conducted on 50 animals from seven species to evaluate pulmonary pathology and enhance understanding of respiratory disease processes. Of the 50 necropsied cases, 35 exhibited lung lesions of varying etiologies, distributions, and severities. The affected animals included 18 dogs, 8 cats, 3 chickens, 3 goats, 1 swine, 1 equine, and 1 Asian water monitor. Notably, no prior history of respiratory disease was reported in these cases, underscoring the role of necropsy in uncovering subclinical or undiagnosed pathology. Lesions were anatomically classified into four major categories: airwaycentered (bronchitis, peribronchitis, aspiration pneumonia), alveolar/parenchymal (interstitial pneumonia, granulomatous pneumonia, emphysema, atelectasis, fibrosis, osseous metaplasia), vascular (heartworm-associated lesions, pulmonary congestion, pulmonary edema), and pleural (pleurisy). Severity ranged from acute and reversible conditions to chronic, fibrosing, or degenerative changes. Gross lesions were varied: pulmonary congestion, frothy exudates, parasitic infestation, aspiration pneumonia, and lung consolidation, further characterized by histopathology, revealing inflammatory cell infiltration, alveolar septal thickening, vascular thrombosis, parasitic structures, and fibrotic remodeling. Special histochemical stains—Alcian blue, PAS, Masson's trichrome (MTC), and Gram —were utilized selectively. These findings emphasize the diagnostic value of postmortem examination supported by histopathology in detecting and differentiating pulmonary disease, and they highlight the diversity of respiratory pathology across species.

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Optimizing Maropitant Transdermal Delivery: Synthesis and Invitro Evaluation of Solid Lipid Nanoparticles

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Transdermal delivery systems for drugs have revolutionized therapeutic approaches by driving drugs through the skin non-invasively and into circulation. Such systems offer extended and controlled drug delivery, comfort for the patient, reduced dosing frequency, and enhanced drug stability. By circumventing hepatic first-pass metabolism, the plasma concentrations of drugs are enhanced by transdermal agents. This study involves the synthesis of solid lipid nanoparticles (SLNs) of Maropitant for delivery through the skin in a transdermal manner. Maropitant, used in animal drugs as an anti-emetic for canines and felines, has conventionally been administered as a pill or in injectable dosages. We utilized the injectable form in preparing SLNs, which include a surfactant outer surface and lipoid core with drug dissolved in it. Such nanoparticles containing drugs can be delivered through the skin and offer drugs by sustained delivery. The methods used in the study include ultrasonication, emulsification using solvent, extrusion, sonication, freeze-drying, and gelation. We will examine particle size, zeta potential, encapsulation efficiency, and in vitro diffusion using Franz cells with Strat-M membrane. This research aims to expand transdermal formulations for cats, addressing Maropitant's high molecular weight and improving compliance and dosing frequency. Lipoid matrix mimics lipids in the feline skin and enhances penetration and diminishes irritation.

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Gremlin1 Overexpression in Nephron Progenitors Impairs their Maintenance and Differentiation into Nephrons

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Approximately 35.5 million Americans suffer from chronic kidney disease, with variation in nephron number being a potential predisposing factor. Therefore, it is critical to define the mechanisms that govern the renewal and differentiation of nephron progenitor (NP) cells into nephrons. Gremlin 1 (GREM1), a BMP signaling antagonist, is a critical protein in kidney development and function. Global deletion of GREM1 in mice leads to kidney agenesis. However, the consequences of overexpression of GREM1 in NP lineage remain unknown. We hypothesized that GREM1 overexpression in NP lineage will disrupt nephrogenesis. To test this hypothesis, we generated conditional mutant mice in which GREM1 expression was induced in Six2+ve cells and their derivatives. Six2-Cre-GREM1 mutant mice at 3-wks of age had significantly decreased kidney weight to body weight ratio and fewer nephrons. Histological analysis showed that mutant mice had impaired glomerulogenesis and dilated tubules. Mutant kidneys showed reduced size at postnatal day 1 (P1), with dilated tubules and impaired glomerular tuft formation. Immunofluorescence (IF) analysis revealed disrupted development across all nephron segments. Markers for NP (ITGA8) and ureteric buds (UB) (TROMA1) showed a reduction in NP caps and UBs. NP-derived epithelial structures such as renal vesicle and S-shaped body that express NCAM1 and WT1 were fewer in the mutant vs control. Co-IF with markers of podocyte (WT1), mesangial (GATA3), and endothelial (CD31) cells indicated defective glomerular tuft development. Together, our results show that X overexpression in NP lineage impairs renal development by disrupting NP maintenance and differentiation into all nephron segments.

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