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Letter from the director

Dear colleagues and friends,

Welcome to the 18th annual University of Florida Emerging Pathogens Institute Research Day!

We have increased our numbers from the previous year to include nearly 150 abstract submissions, all demonstrating the breadth of research that highlights the multidisciplinary focus that makes our institute great. This year, our poster session boasts remarkable work from nine UF colleges and 33 UF departments, ranging from biomedical engineering to African studies to neurosurgery. In addition, we also have presentations from collaborators at state and federal agencies.

Furthermore, we've expanded our poster competition to include undergraduate students — the future generation of infectious disease researchers. We encourage you to make new connections and make this another unforgettable event as you participate in it.

We have the honor of introducing our speaker, Dr. Alberto Paniz-Mondolfi. Mondolfi is an internationally recognized pathologist from the Icahn School of Medicine at Mount Sinai who will talk about the hunting for microbial diversity in Latin America.

I remember attending my first research day back in 2009. Now, as interim director, it's wonderful to be a part of this event still almost two decades later. I look forward to seeing all of you for what is going to be a very exciting day.

Best,
Marco Salemi, Ph.D.
Interim Director and Professor

Schedule of events

9:00 AM

Breakfast & Registration

9:50 AM

Welcome & Introductions

10:00 AM

Poster Session

12:00 PM

Lunch & Raffle Prizes

1:45 PM – 2:45 PM

Keynote Speech

3:10 PM

Competition Winners Announcement

3:20 PM – 3:30 PM

Closing Remarks

3:30 PM – 4:00 PM

Poster Removal

1:45 p.m. – 2:45 p.m.

Alberto Paniz-Mondolfi, M.D., Ph.D.

Associate Professor
Icahn School of Medicine at Mount Sinai
New York, USA

"The hunt for pathogens: Exploring microbial diversity in Latin America."

01. A DATA-FOCUSED ANALYSIS OF LEAFY GREEN HARVEST EQUIPMENT CLEANING & SANITATION PRACTICES

LaTaunya Tillman - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Clara Diekman** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Dalton Zingali**; **Trevor V. Suslow**; **Justin Kerr**; **Channah M. Rock; Michelle Danyluk** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: Field harvesting and packing equipment have been implicated as potential sources of microbiological contamination; equipment complexity and variations in use have led to varied cleaning and sanitizing practices.

Purpose: Assess leafy green harvester cleaning and sanitation programs to identify opportunities for improvement.

Method: An assessment of cleaning and sanitation practices used for leafy green harvest machines was completed across three states: AZ, CA, and FL. Self-Propelled Belt and 3-Point harvesters were sampled for ATP, Aerobic Plate Count (APC), Total Coliform (TC), and Generic E. coli (gEC) at three time points, Post-Harvest(PH), Post-Detergent(PD), and Post-Sanitation(PS). Harvest machine sampling locations were standardized across events, with each machine having up to five locations. Metadata was recorded for each sampling, including practices, location, equipment type, condition, cleaning accessibility, surface type, environmental data, and parameters that could have impacted sample outcomes. Swab

samples were analyzed using AOAC standardized methods; T-tests and ANOVAs were performed (n=30).

Results: Leafy green harvest machines (23 machines) were assessed over 30 sampling events across three states (11-AZ, 9-CA, and 10-FL). Considerable variability was observed in detergents, sanitizers, crew sizes, and water usage. PH, 94% of swabs across all areas were above the ATP threshold (20,000 RLU; n=660); this decreased to 47% of swabs after sanitation (PS)(n=382). E. coli detection (⊠10 CFU/swab) was infrequent and decreased after cleaning and sanitizing: PH, 6.2%; PD, 5.3%; PS, 1.1%. TC reductions after cleaning (n=493) and sanitizing (n=208) ranged from -3.54 to 6.13 log CFU/swab; occasionally, TC populations increased after cleaning (36.1%) and sanitizing (18.3%) compared to swabs immediately following harvest. Significant differences ($p<0.05$) between detergent and sanitizer combinations were observed in ATP, APC, TC, and gEC reductions.

Conclusion: Practices and challenges were similar across all states, and opportunities (sometimes very simple) exist for improvement. Detergent type, application, and equipment used during cleaning had a greater impact on microbial reduction than sanitizers alone.

02. ACCURACY AND PERFORMANCE OF LONG READ VERSUS SHORT READ SEQUENCING PLATFORMS USING ESCHERICHIA COLI RECOVERED FROM FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) AS A BENCHMARK ORGANISM

Frank J. Tuozzo II - Department of Microbiology and Cell Science, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Morgan C. Metrailer** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Andrew Bluhm** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **Treenate Jiranantasak** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida; **An-Chi Cheng** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Juan M. Campos Krauer** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Samantha Wisely** - Department of Wildlife Ecology and Conservation, College of Agricultural and Life Sciences, University of Florida; **Jason K. Blackburn** - Department of Geography, Emerging Pathogens Institute, College of Liberal Arts and Sciences, University of Florida

Escherichia coli is a widely used and easily accessible bacteria frequently found in almost all warm-blooded organisms. Due to its well-understood and easily manipulatable genome, rapid cultivation, safety, and compatibility with many modern sequencing technologies, E. coli has been used as a benchmark for sequencing studies for decades. MinION is a palm sized, field-ready sequencer designed by Oxford Nanopore Technologies, that produces rapid results and can sequence short to ultra-long fragments. Due to this system's focus on swift in-the-field sequencing, the single nucleotide polymorphism (SNP) accuracy

with rapid base calling has been unsatisfactory; SNPs are essential for many phylogenetic analyses. The new Dorado base calling software has reported higher accuracy in SNP calls, suggesting SNP results closer to Illumina's more accurate short read platform. Here, whole genome sequencing utilizing the MinION Native Barcoding Sequencing Kit 24 applying both high-accuracy, super-accurate, and fast base calling methods, as well as Illumina sequencing were performed at the University of Florida. The E. coli isolates used for this comparative analysis were sampled from farmed Florida white-tailed deer mortalities as part of the University of Florida Cervidae Health Research Initiative (CHeRI). The sequence data were assembled and analyzed using UF's High Performance computer (HiPerGator). From this, multiple typing schemes that utilize varying portions of the genome were performed including: phylogroup typing, gene identification, as well as cgMLST and SNP-based tree building. These datasets were then used for comparative analyses between the two sequencing platforms. Results suggest that using MinION's higher accuracy methods rather than fast base calling method improves accuracy closer to Illumina's short-read sequencing, suggesting the MinION may be an effective, lower cost option to quickly type samples, including field settings.

03. BIOLOGICAL SOIL AMENDMENTS OF ANIMAL ORIGIN AND ENVIRONMENTAL FACTORS ALTER SOIL MICROBIOME AND PATHOGEN PERSISTENCE

Yuting Zhai - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida; **Cameron Bardsley** - U.S. Department of Agriculture; **Karuna Kharel** - Loisanna State University; **Charles B. Appolon** - University of Georgia; **Manan Sharma** - U.S. Department of Agriculture; **Laurel L. Dunn** - University of Georgia; **Michelle Danyluk** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Keith R. Schneider** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Soil microbiomes regulate nutrient cycling, pathogen suppression, and plant productivity in agroecosystems. Biological soil amendments of animal origin (BSAAO) are able to improve soil fertility but may also prolong pathogen persistence, raising food safety concerns. This study investigated the impact of BSAAO application on soil microbiomes and its influence on Escherichia coli survival. Microbiome shifts were analyzed over 140 days in Florida and Georgia soils treated with composted poultry litter, heat-treated poultry pellets, or left unamended. Despite differences in native soil microbiomes, BSAAO significantly altered microbial composition, enriching taxa associated with nutrient cycling and antimicrobial production. Machine learning identified an Escherichia-Shigella-associated OTU positively correlated with E. coli persistence and negatively associated with soil moisture. These findings reveal how BSAAO-driven microbial shifts influence

E. coli persistence, emphasizing the need for microbiome-informed strategies that enhance microbial competition, optimize soil amendments, and reduce pathogen survival to improve food safety in agricultural systems.

04. CELLULAR IMPACT OF MACROPHAGE-DERIVED SMALL EXTRACELLULAR VESICLES FOLLOWING SALMONELLA INFECTION: ROLE OF THE EFFECTOR PROTEIN SSEL

Alex Schultz - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida; **Mariola Ferraro** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Macrophages are key targets of Salmonella Typhimurium, a gram-negative intracellular pathogen. Although innate immune cells, macrophages can act as a "Trojan horse" during Salmonella infection, as the bacterium evades intracellular defenses and facilitates dissemination. Macrophages produce small extracellular vesicles (sEVs), including exosomes, which play important roles in cell-to-cell communication. Our previous studies revealed that sEVs derived from infected macrophages contain proteins, lipids, and foreign antigens, with Salmonella actively modulating their biogenesis by yet unknown mechanisms. While multiple sEV biogenesis pathways are identified in macrophages, the mechanisms by which Salmonella influences sEV formation remain unclear. We hypothesize Salmonella utilizes SseL to manipulate the lysosomal pathway and reduce sEV production in macrophages following infection. Nanoparticle tracking analysis of sEV (-) (uninfected macrophages), sEV (WT +) (macrophages infected with wild-type S. Typhimurium), and sEV (ΔsseL +) (macrophages infected with ΔsseL S. Typhimurium) revealed a

drastic increase in these sEV depending on the SseL. The sEVs (WT +) were more numerous than sEVs (-), and the size of sEV (WT +) and sEV (ΔsseL +) was smaller. Western blots comparing sEV (WT +) and sEV (ΔsseL +) showed little difference in CD63/CD9 expression, however, compared to sEV (-) the expression is significantly decreased. To assess the proinflammatory response elicited by sEVs, naïve macrophages were treated with sEV(-), sEV(WT+), and sEV(ΔsseL+). Supernatants collected at 2 and 24 hours post-infection (hpi) showed that sEV(WT+) and sEV(ΔsseL+) induced strong TNF-α responses, while sEV(-) did not. Mass spectrometry analysis of whole-cell lysates from sEV-treated macrophages identified significant differences in protein abundance, with marked disparities between cells treated with sEVs from infected macrophages (WT or ΔsseL) and those treated with sEV[-]. Macrophage uptake of sEVs was further evaluated using DiR-labeled sEVs, followed by imaging for 24 hours. Results showed that sEVs from infected cells (sEV[WT +]) were more rapidly degraded than those from uninfected cells (sEV[-]). Overall, these findings suggest SseL strongly affects the quantity of sEV production but likely has a limited role in altering their properties, such as size, protein markers, or inflammatory potential. However, if SseL limits the sEV production, it is likely to play important role in the regulation of host cell-to-cell communication.

05. CHARACTERIZING THE ROLE OF CHEMOTAXIS GENES IN SALMONELLA MOTILITY AND TUMOR-TARGETING APPLICATIONS

Isaac Bastos-Cowley - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Chemotaxis, the directed movement of bacteria in response to chemical gradients, is essential to Salmonella's ability to locate and colonize specific environments. Tumor microenvironments, characterized by hypoxia and necrosis, produce chemical gradients that mimic conditions naturally attracting Salmonella. These environments often include amino acids and sugars, such as aspartate, serine, ribose, and galactose, which serve as critical attractants. While Salmonella's tumor-targeting properties are well-documented, the use of individual chemotaxis genes—tar, tsr, and trg— to increase the tumor targeting ability of Salmonella remains developed. This study addresses this gap to optimize Salmonella's potential as a vehicle for targeted cancer therapies.

The study investigates how mutations in tar, tsr, and trg affect Salmonella's chemotactic efficiency. Wild-type and mutant strains were tested using motility assays on agar plates supplemented with specific attractants. Cultures were normalized to consistent optical density (OD600 = 0.9–1.0) to ensure uniform bacterial concentrations across replicates. Motility was quantified by measuring swimming and swarming diameters. Statistical analyses, including one-way ANOVA with Dunnett's post-test, compared mutant strains to the wild type. CFU calculations confirmed consistent bacterial numbers in all experiments.

Preliminary findings suggest that mutations in these genes may alter chemotactic efficiency, with distinct roles observed in

nutrient-specific motility. These results advance our understanding of Salmonella chemotaxis and its potential for tumor targeting. Future research will validate these findings, explore combined gene deletions, and examine interactions between chemotaxis and other tumor-targeting mechanisms.

06. COMPARING METHODS AND DURATION OF AIR SAMPLING TO QUANTIFY BACTERIAL POPULATIONS IN BIOAEROSOLS

Christina Kessler - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Keith R. Schneider** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida; **Michelle Danyluk** - Department of Food Science and Human Nutrition, College of Agricultural and Life Sciences, University of Florida

Introduction: Bioaerosols from adjacent animal operations have been identified as a potential risk for produce contamination. Isolation of foodborne pathogens and indicators from air can be challenging.

Purpose: To compare active and passive sampling methods to detect foodborne pathogens and indicators from air surrounding an animal operation.

Methods: Air samples were collected (n=6) near an animal operation. Active samples were collected onto Brilliance E. coli/coliform (BEC), and Salmonella (CS), and Shiga toxin-producing E. coli (STEC, CX) chromogenic agar using Andersen impact samplers (5, 10, and 20 min). Plates were incubated at 37°C for 48h. Passive air sampling was performed using cheesecloth filters (12 and 24h). Cheesecloth was stomached with 200ml 2% buffered peptone water (BPW) + 0.2% tween80, plated onto tryptic soy agar (TSA) and BEC, and incubated at 37°C for 24h. Remaining

rinsate was combined with double-strength tryptic soy broth (TSB), incubated at 37°C for 24h, and streaked onto Sorbitol MacConkey (SMAC) agar and CX for STEC detection. For Salmonella detection, 9.0ml tetrathionate (TT) and 9.9ml Rappaport Vassiliadis (RV) broth were inoculated with 1.0 and 0.1ml TSB enrichment, respectively, and incubated at 42°C. After 24h, broths were streaked onto hektoen enteric (HE) agar, xylose lysine deoxycholate (XLD) agar, and CS. STEC and Salmonella suspect isolates were confirmed via qPCR and the presence of stx1/stx2 and invA genes, respectively.

Results: Increasing active sampling time (5, 10, and 20 min) decreased coliforms (<0.77, <0.19, and <-0.47 log CFU/m3, respectively) and generic E. coli counts (<0.74, <0.24, and <-0.44 log CFU/m3, respectively). Salmonella and STEC were not detected. Higher bacterial populations were seen at 12 vs 24 h during passive sampling: total counts (0.53 vs 0.24 log CFU/cheesecloth/h), coliforms (0.27 vs 0.13 log CFU/cheesecloth/h), and E. coli (<0.23 vs <0.10 log CFU/cheesecloth/h). Out of 6 passive 12h samples, 4 tested positive for Salmonella in at least one enrichment/media combination. STEC was not detected.

Conclusion: Findings highlight the impact of sampling strategies on assessing potential risks of bioaerosol-mediated contamination in produce environments.

07. COMPARISON OF MOLECULAR APPROACHES TO DETECT VIRULENT BACTERIOPHAGE AS A PROXY FOR V. CHOLERAE

Michelle Mu - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Ishae Sriguha** - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Sharia Ahmed** - Department of Epidemiology, Emory University; **Md Abu Sayeed** - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Emilee Cato** - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Ashton Creasy-Marrazzo** - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida; **Firdausi Qadri** - Infectious Diseases Division, International Centre for Diarrhoeal Disease Research; **B. Jesse Shapiro** - Department of Microbiology & Immunology, McGill University; **Ashraful Islam Khan** - Infectious Diseases Division, International Centre for Diarrhoeal Disease Research; **Eric Nelson** - Department of Pediatrics and Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida

Introduction: Cholera is a diarrheal disease caused by the Gram-negative water-borne pathogen Vibrio cholerae. Globally, there are 1.3-4 million reported cases annually resulting in 21,000-143,000 deaths. Methods to detect V. cholerae and related enteric pathogens include culture, polymerase chain reaction (PCR) and quantitative PCR (qPCR). Nano-liter qPCR (nl-qPCR) is an alternative to qPCR that utilizes a high-throughput platform of nanofluidic volumes and cuts costs six-fold. PCR-based molecular approaches offer faster, more sensitive and more specific results

than culture. The primary objective of this study was to compare different modes of molecular detection of V. cholerae and its associated virulent bacteriophages (phages). The secondary objective was to compare associated costs.

Method: We conducted three different molecular detection assays (PCR, qPCR, nl-qPCR) on 2574 stool samples collected from patients admitted with diarrheal disease in Bangladesh. Targets included V. cholerae and its phages (ICP1/2/3). To evaluate the pairwise agreement, we used Cohen's kappa. We conducted McNemar's test to compare qualitative results. Associated costs and comparisons were analyzed based on a per reaction cost (cpr); cost of machinery was excluded.

Results: Comparison between qPCR and nl-qPCR showed strong agreement for tcpA (for V. cholerae, κ=0.886) and moderate agreement for ICP1 (κ=0.767), ICP2 (κ=0.768), and ICP3 (κ=0.597). Three-way analysis among PCR, qPCR, and nl-qPCR showed strong agreement for V. cholerae (κ=0.785) and moderate agreement for ICP1 (κ=0.609), ICP2 (κ=0.593), and ICP3 (κ=0.533). McNemar's test showed evidence of statistically significant differences in the marginal probabilities of a positive detection by qPCR and nl-qPCR for the targets tcpA and ICP3, but no significant evidence for differences in detection of ICP1 or ICP2. With respect to cost, PCR was most expensive ($1.31 cpr) compared to qPCR ($1.05 cpr) and nl-qPCR ($0.004 cpr); cost attribution for PCR was both the gel matrix and reaction mixture.

Conclusions: The different molecular approaches taken herein showed better congruence for V. cholerae compared to detection of associated phages. Although qPCR and nl-qPCR require more complex machinery, they offer lower costs per sample, increasing scalability when the machinery is pre-existing. These results support both qPCR and nl-qPCR in diagnostic approaches for

detecting V. cholerae, as well as its phages as a proxy for pathogen detection. These results may have impact on related diseases that are less tractable to study.

08. CONTROL OF MURINE NOROVIRUS INFECTION BY BACTERIAL EXTRACELLULAR VESICLES OCCURS THROUGH DNA MEDIATED IMMUNE RESPONSES

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Human noroviruses are the leading cause of gastrointestinal disease worldwide and are responsible for most diarrheal death in developing nations. Despite the global health burden caused by the pathogen relatively little is known about the mechanisms of norovirus pathogenesis. However, recent studies have shown that commensal bacteria within the gut have the capacity to suppress

and enhance viral replication in a region-specific manner. Commensal bacteria inhibit viral replication in the proximal small intestine, while simultaneously enhancing viral replication in the distal end. Our findings have shown that norovirus interacting with commensal bacteria lead to the increased production of bacterial extracellular vesicles (bEVs) in vitro and vivo providing the opportunity for these vesicles to influence viral infection. Recently, it has been proposed that commensal bEVs can prime the host with cGAS-STING mediated type I IFNs to suppress systemic HSV infection. However, the local effect of these commensal bEVs on macrophages during an enteric RNA virus infection has yet to be determined. Therefore, we have now begun characterizing the impact of bEVs on various aspects of the norovirus infectious cycle, and we hypothesized that these bEVs can prime macrophages with antiviral type I IFNs via the cGAS-STING pathway to limit MNV replication. Our results show that bEVs can enter RAW macrophages quickly (15 minutes). Treating RAW macrophages with bEVs and MNV-1 has resulted in reduced cell cytotoxicity and bEV pretreatment in mice suppressed MNV replication across multiple sections of the intestines. RT-qPCR data shows that STING mediated pathway is being induced to mediate antiviral immune response. Luciferase assay also indicates that a STING KO construct failed to produce as much type I interferons compared to the WT. Using single cell RNA-seq, we are able to get a complete view of the impact of bEVs and the possible mediated pathways. Understanding how bEVs induce the host to mediate unconventional antiviral pathways to suppress viral replication could provide a mechanistic framework for the identification of targets to prevent or treat norovirus infection.

09. DETERMINING THE ROLE OF OMV-ASSOCIATED BACTERIAL SPHINGOLIPIDS IN MODULATING MURINE NOROVIRUS INFECTION IN MACROPHAGES

Elysia Vivanco - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida; **Joseph Sullivan** - Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida; **Melissa Jones** - Department of Microbiology and Cell Science, Emerging Pathogens Institute, College of Agricultural and Life Sciences, University of Florida

Although human norovirus (HuNoV) is the predominant cause of acute gastroenteritis worldwide, effective treatments have yet to be developed against it. Both human and murine noroviruses (MNV) directly engage with the commensal bacteria of the intestinal tract, leading to changes in bacterial gene expression. We have previously shown that the commensal bacterium Bacteroides thetaiotaomicron increases production of outer membrane vesicles (OMVs) in response to this bacterial-noroviral interaction. Murine-derived macrophages exhibit heightened immunity against MNV when exposed to these OMVs; however, the specific component(s) within the OMVs that contributes to this immune response is unknown. In this study, we investigated the ability of B. thetaiotaomicron OMV-associated sphingolipids—a class of lipids with immune-signaling capabilities—to suppress MNV replication in murine macrophages. Macrophages were co-inoculated with MNV and OMVs generated from wild-type B. thetaiotaomicron or sphingolipid-deficient Δspt mutant B. thetaiotaomicron. Quantification of viral replication using RT-qPCR revealed that significantly more MNV replication occurred in samples treated with Δspt OMVs compared to wild-type OMVs. Notably, neither wild-type nor Δspt OMVs substantially limited viral replication in

KO-TLR4 macrophages. Analysis of mRNA expression via RT-qPCR has shown that genes involved in innate immunity (ifnb1, mcp1, and isg15) are upregulated in wild-type OMV-treated cells, but the difference compared to cells treated with Δspt OMVs is insignificant when MNV is present. Together, these findings demonstrate that OMVs containing sphingolipids play a role in modulating norovirus infection, with a dependence on the TLR4 signaling pathway. Understanding which component induces the immunologic response against norovirus can potentially aid in the development of novel therapeutics to treat or prevent human norovirus infection.

10. DEVELOPMENT OF TUMOR-TARGETING PLASMIDS FOR SALMONELLA-BASED CANCER THERAPY

Yu Wei - Department of Infectious Disease, College of Veterinary Medicine, University of Florida; **Shifeng Wang** - Department of Infectious Disease, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida

Salmonella, known for its facultative anaerobic nature to replicate in tumors and ability to deliver foreign genes into tumors, is a promising candidate for anticancer therapies. However, clinical trials showed that the targeting ability needs to be strengthened. This study focuses on developing tumor-targeting Salmonella vectors. Plasmids carrying targeting peptides were generated through a systematic approach involving vector enzyme digestion, PCR amplification of target fragments, agarose gel electrophoresis, DNA element extraction, DNA quantification, Gibson assembly (a molecular cloning technique that joins multiple DNA fragments in a single reaction) of the vector and target fragment, preparation of electrocompetent cells, bacterial transformation, recombinant plasmid screening, and plasmid verification using PCR and sequencing. The plasmids also have a gene encoding fluorescent

protein GFP. As a reporter gene, it presents as an operon fusion with the target peptide, both of them are under the control of the same Ptrc promoter. A total of 12 different peptides targeting either ovarian cancer or melanoma were generated. Sequencing confirmed the presence of the correct DNA fragments within the constructed plasmids. Currently, these plasmids have been transformed into suitable Salmonella strains. Future steps will assess the ability of the Salmonella strains with these plasmids to target and interact with specific cancer cells. This research represents a novel approach to bacterial-mediated cancer therapy, offering potential breakthroughs in targeted treatment strategies.

11. ENGINEERED POLYSACCHARIDE A NANOPARTICLES FOR INFLAMMATORY BOWEL DISEASE THERAPIES

Zhenyu Wang - Department of Biomedical Engineering, College of Engineering, University of Florida; **Nicole Crow** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Julia Leser** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Jada Brown** - Department of Biomedical Engineering, College of Engineering, University of Florida; **Rian Harriman** - Department of Biomedical Engineering, University of California, Davis; **Jamal S. Lewis** - Department of Biomedical Engineering, College of Engineering, University of Florida

Inflammatory bowel disease (IBD) is a series of chronic inflammatory events in the gastrointestinal tract including Ulcerative Colitis and Crohn's disease. These diseases, which affect >3 million Americans, inflict serious health burdens and financial costs (estimated $500M annually) (Kahn-Boesel et. al, 2022), and broadly interfere with patients' daily lives. Existing therapeutic interventions mainly focus on anti-inflammatory drugs, especially small-molecule drugs (e.g., aminosalicylic acid), and monoclonal

antibodies targeting inflammatory-related factors (e.g., infliximab) (Cai et. al, 2021). However, they are commonly limited by short-term efficacy and unwanted adverse side effects such as increased risk of infection and cancer. Thus, there is an urgent need to develop an alternative IBD treatment that is effective and targeted. In the past few decades, it has been revealed that commensal bacteria play essential roles in regulating the host immune system from abnormal inflammatory responses via different mechanisms including but not limited to specific immunomodulatory molecules, such as Polysaccharide A (PSA) (Erturk-Hasdemir et. al, 2018). PSA is a capsular molecule that is found in the gut commensal bacteria, Bacteroides fragilis (B.F.), and has been reported to show great therapeutic potential in many mouse models of inflammation, due to its ability to induce regulatory T cells (Tregs) (Ramakrishna et. al, 2019; Ochoa-Reparaz et. al, 2010). The tolerogenic properties, accompanied with its polymeric structure, qualify PSA as a promising biomaterial for the formulation of tolerogenic nanoparticles. Here, we hypothesized that PSA nanoparticles (PSA NPs) encapsulating an IBD-relevant autoantigen - ovalbumin (OVA), will be a more efficacious and safer therapy due to the PSA NP's ability to induce OVA-specific Treg differentiation and to protect the OVA from mast cell activation mediated by IgE receptor. Overall, we hypothesized that this tolerogenic nanoplatform could promote antigen-specific tolerance by activating dendritic cells (DCs) to induce Treg differentiation, ultimately alleviating inflammation associated with IBD. Currently, PSA has been successfully extracted from bacteria following an optimized extraction protocol, and its purity and activity have been testified via NMR and mTLR2 Test. Furthermore, the PSA's tolerogenic effect on DC phenotype has been studied in two different subsets of DCs through flow cytometry. The qualified PSA was then used for PSA NP fabrication

and its physicochemical properties including size and surface charge are characterized via Dynamic Light Scattering (DLS), which provides a robust framework for the development of the tolerogenic platform.

12. EXPLORING TRANSCRIPTOMIC CHANGES IN ANTIBIOTIC-RESISTANT SALMONELLA HEIDELBERG MODULATED BY COMMENSAL ESCHERICHIA COLI IN VITRO

Yasir Razzaq Khan - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Lekshmi Edison** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida; **Thomas Denagamage** - Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida; **Subhashinie Kariyawasam** - Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida

The nontyphoidal Salmonella (NTS) enterica serotype Heidelberg is a major foodborne pathogen primarily transmitted to humans through contaminated poultry products. In recent years, the increase in antibiotic-resistant NTS, including Salmonella Heidelberg, has become an additional public health concern. The effectiveness of current control measures emphasizes the need for novel approaches to mitigate NTS colonization in poultry and contamination of poultry products, thereby reducing its transmission to humans. We hypothesized that commensal Escherichia coli can reduce antibiotic-resistant NTS colonization in the chicken intestines by modulating fitness, virulence, and antibiotic resistance potential of Salmonella. As the first step to explore the effect of commensal E. coli on antibiotic-resistant NTS and vice versa in vitro, we co-cultured commensal E. coli 47-1826 and antibiotic-resistant S. Heidelberg 18-9079 strains of poultry

origin in Luria Bertani broth and performed in-depth analysis of their transcriptomes using high-throughput RNA sequencing. This study identified 4,890 differentially expressed genes in S. Heidelberg 18-9079 when co-cultured with E. coli 47-1826. After expression data filtration, of these 4,890 differentially expressed genes, 193 and 202 showed significant upregulation and downregulation, respectively. Notably, several genes involved in bacterial growth, pathogenicity and virulence, biofilm formation, metal-ion hemostasis, signal transduction and chemotaxis, stress response, transmembrane transport of xenobiotics, and cellular metabolism were down-regulated up to eighty-six folds in S. Heidelberg as compared to S. Heidelberg control. Further, the study revealed downregulation of genes involved in antibiotic resistance and drug efflux in S. Heidelberg 18-9079 up to twelve folds. These findings highlight that commensal E. coli 47-1826 can potentially reduce the fitness, persistence, virulence, and antimicrobial resistance (AMR) dissemination of S. Heidelberg 18-9079 and be used to mitigate antibiotic-resistant S. Heidelberg in poultry to enhance food safety.

13. GENETICALLY ENGINEERING PROBIOTIC E. COLI NISSLE 1917 TO EXPRESS NOROVIRUS VP1 CAPSID PROTEIN IN ITS OUTER MEMBRANE VESICLES

Alissa Katsaras - Department of Microbiology and Cell Science, College of Agricultural and Life Sciences, University of Florida;
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Norovirus is a positive sense single stranded RNA virus of the Calciviridae family, most widely known for its role as the leading cause of acute gastroenteritis worldwide.1,2 Human norovirus (HuNov) has been responsible for consistent outbreaks since its emergence, with an average of up to 800 deaths and 71,000 hospitalizations per year, just within the United States.3 These cases disproportionately affect children under 5 and adults over 85, with the majority of fatalities occurring in developing countries.3 Despite the global burden of norovirus, specifically on vulnerable communities, a successful vaccine has yet to be developed. The ongoing challenges in developing a vaccine for norovirus can be attributed to the continuous emergence of new strains due to genetic/antigenic drift, the high plasticity of the VP1 capsid protein, and the virus's exceptionally high rate of spontaneous mutation.4 In addition, the inability of natural infection to elicit long-term protective immune responses is another significant hurdle for norovirus vaccine development.5 In order to work towards the development of a successful norovirus vaccine, the delivery of norovirus antigens and proteins to immune cells must further be investigated. Outer membrane vesicles (OMV's) have been identified by recent studies as a prospective vector for vaccination.6 These nanoparticles are excreted from the cellular

membrane of gram-negative commensal bacteria and are composed of lipids, proteins, and other biomolecules.7 OMV's are being examined as a potential vaccine vector due to the presence of immunogenic surface proteins that induce an immunomodulatory response in the host. The genetic modification of bacteria to produce OMV's that express exterior viral proteins as a method of vaccination is the leading concept behind this investigation. E. coli Nissle 1917 (EcN) is a gram-negative bacterial strain recognized for its probiotic capabilities such as suppression of immune-mediated damage and other immunotherapeutic pathways.8 EcN is a good candidate for the genetic modification of its OMVs since it is well-studied, has established immunomodulatory effects, and enhances the gut microbiome. The results of this study will have major implications for the future of norovirus vaccination through the use of engineered nano vesicles.

14. GENOMIC COMPARISON AND GENETIC CHARACTERISTICS ASSOCIATED WITH HOST SPECIFICITY OF SALMONELLA ENTERICA SEROVAR DUBLIN USING MACHINE LEARNING

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Salmonella enterica serovars pose a significant public health threat due to their high morbidity and mortality rates, compounded by the increasing prevalence of antimicrobial resistance (AMR). Among these, S. enterica serovar Dublin (S. Dublin), a cattle-adapted serotype, is becoming increasingly prevalent in dairy cows, where it is associated with respiratory disease and septicemia. Additionally, S. Dublin is a zoonotic pathogen, presenting a notable risk to human health. This study aimed to investigate the genomic characteristics of S. Dublin isolates from humans, animals, and environmental sources to identify host-associated genetic features using machine learning (ML). A total of 200 S. Dublin genomes, including publicly available sequences and those sequenced in-house, were analyzed. These isolates exhibited diverse antimicrobial resistance gene (ARG) profiles, including resistance to tetracycline, fluoroquinolone, sulfonamide, aminoglycoside, and β-lactam antibiotics, highlighting the presence of multidrug resistance. All isolates also harbored virulence factors linked to adherence, invasion, iron uptake, and Type III secretion systems, underscoring their pathogenic potential. Using a support vector machine (SVM) ML model, we analyzed the whole-genome sequences to predict the host source (human vs. non-human) of S. Dublin isolates with high accuracy. Notably, host-specific genetic markers were identified, including the ygdG gene, which facilitates glucose transport across bacterial membranes; the oadG gene, which converts oxaloacetate to pyruvate while supporting sodium ion transport; and the hdfR gene, which regulates motility and stress-response pathways. These genes appear to play critical roles in host adaptation, shedding light on the mechanisms driving host-specific interactions in S. Dublin.

15. IN VITRO EVALUATION OF POTENTIAL PROBIOTIC BIFIDOBACTERIUM LONGUM ISOLATED FROM FECES OF DAIRY CALVES

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Bifidobacterium, known for their beneficial effects, are commonly used as probiotics in humans due to their ability to inhibit colonization by harmful pathogens such as Escherichia coli and Salmonella. These pathogens contribute to diarrhea, sepsis, and other critical diseases that significantly affect the mortality rate of dairy calves. Antibiotics are commonly used in treating animal disease, however, it raised a concern of emerging antibiotic resistance in animals. Here we aimed to select B. longum strains as probiotic candidates for treating calf diarrhea. Therefore, we hypothesized that Bifidobacterium longum strains could survive in the gastrointestinal environment and suppress pathogen colonization. Fifty-four strains of B. longum were isolated from feces of healthy calves aged 3 to 28 days and identified using whole genome sequencing. Consequently, twelve B. longum strains were selected as probiotic candidates. In vitro assays, including simulated intestinal fluid (SIF) tolerance, acid tolerance, bile salt tolerance, and lysozyme tolerance, were performed to assess their survivability in gastrointestinal conditions. All B. longum strains survived in SIF containing pepsin at pH 2, although viability varied among strains. They were also able to survive in growth media containing low pH, bile salts, or lysozyme, indicating that B.

longum strains possess the ability to survive gastrointestinal conditions. Antimicrobial activity was assessed to evaluate their ability to suppress pathogen growth using soft agar inoculated with pathogens such as E. coli and Salmonella. Supernatants of B. longum were placed into wells on soft agar plates, and clear inhibition zones formed around the wells, demonstrating their antimicrobial capabilities against pathogens. Additionally, adhesion and colonization abilities of B. longum strains were evaluated through hydrophobicity and auto-aggregation assays. Hydrophobicity and auto-aggregation rates differed among strains, indicating that they have distinct characteristics despite belonging to the same species. Based on these in vitro assays, B. longum strains demonstrated strong potential to survive in gastrointestinal tracts, suggesting their suitability as effective alternatives to antibiotics for animal treatment.

16. PROBING THE INTERACTIONS BETWEEN COLIBACTIN AND SMALL OLIGONUCLEOTIDES

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Introduction: Colibactin is a genotoxic secondary metabolite produced by pks+ enterobacteria, including specific strains of Escherichia coli, and is implicated in promoting colorectal oncogenesis. Colibactin contains an α-aminoketone functional group, which is highly susceptible to aerobic oxidation, leading to degradation in mild conditions. For this reason, colibactin has never been directly isolated from bacterial cultures and remains elusive.

Method: Due to the challenges associated with isolating naturally active colibactins from E. coli, we employ stable synthetic colibactin analogs to study their interactions with small oligomers.

Results: In this study, we demonstrate that our synthetic colibactin analogs exhibit activity levels comparable to naturally virulent colibactins. They predominantly form mono-adducts and, to a lesser extent, multiple alkylated DNA adducts when interacting with oligomers. Furthermore, MALDI analysis highlights the sequence selectivity of colibactin in forming DNA adducts.

17. RECEPTOR ACTIVITY POST-COXSACKIEVIRUS B3 INFECTION DURING ACUTE MYOCARDITIS

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Introduction: Coxsackievirus B3 (CVB3) is an enterovirus commonly known to cause heart inflammation, or myocarditis. The coxsackie-adenovirus receptor (CAR) mediates cell surface attachment and infection by coxsackieviruses. As part of the host response to viral infection, toll-like receptors (TLRs) play an essential role in innate immune activation through the detection of pathogen-associated molecular patterns on the cell surface to combat the virus. CVB3 is known to activate the immune system during myocarditis through TLR2, 4, and 7.

Hypothesis: This project focuses on observing the relationship of TLRs and CAR to CVB3 and percent inflammation in mice during acute myocarditis. We hypothesized that TLRs and CAR would correlate to CVB3 and myocarditis severity.

Methods: BALB/c male mice were infected with CVB3 at day 0, and mice were harvested at day 10 post-infection during the peak of myocarditis. Half of the heart was used for histological assessment, and half was used for qRT-PCR to quantify relative gene expression levels of Cvb3 VP1 (major capsid protein of CVB3), Tlr2, Tlr4, Cxadr (CAR gene). One-tailed student t-tests were performed between control and myocarditis mice, and one-tailed Pearson's correlation was performed to observe trends between receptor and myocarditis severity (n=6-8/group)

Results: Cvb3 VP1 (p=0.04), Tlr2 (p=0.01), and Tlr7 (p=0.04) were significantly upregulated, and Cxadr (p=0.01) was significantly downregulated during acute CVB3 myocarditis compared to uninfected mice. We found that the higher the Cxadr expression, the lower the Tlr2 and 7 expression and inflammation were (p=0.03, 0.04, and 0.08, respectively), whereas CVB3 VP1 positively correlated to Tlr2 and Tlr7 expression (p=0.04 and 0.02, respectively). CVB3, Tlr2, and Tlr7 were all borderline positively correlated with increased myocarditis severity (p= 0.07, 0.07, 0.05, respectively).

Conclusions: These findings indicate the activation of the innate immune response to the CVB3 infection in mice and the resulting increase in myocarditis severity. This suggests that these receptors could be biomarkers of disease severity and potential therapeutic targets. The downregulation of CAR expression corresponds with an upregulation in TLR expression, potentially indicating immune-mediated suppression of CAR during CVB3 infection.

18. REDUCTION OF NOROVIRUS ON TOMATOES IN AN OVERHEAD SPRAY AND BRUSH ROLLER SYSTEM

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Introduction: Addition of sanitizer to single pass water (i.e. overhead spray) used during produce packing can reduce bacterial foodborne pathogens on the surface of fruits and vegetables; less is known about reduction of viral pathogens.

Purpose: The purpose of this work is to evaluate the reduction of MS2 bacteriophage, a norovirus surrogate, on tomatoes in an overhead spray and brush roller system.

Methods: Unwashed, mature green tomatoes were inoculated with 10, 10 µl spots of ca. 9 log PFU/ml MS2 bacteriophage, acting as a surrogate for norovirus. The inoculated tomato was placed onto a model overhead spray brush roller system, with one uninoculated tomato on either side. The wash system was run for 30 s, with different concentrations (0, 20, 60, and 80 ppm) of a peracetic acid (PAA) mixed into well water. For each PAA concentration, five technical replications with three biological replicates were conducted (n=15 tomatoes). MS2 reductions were evaluated on the inoculated tomatoes. MS2 was recovered from the tomatoes using phosphate buffered saline with 1% sodium thiosulfate and a 30 s shake, rub, shake method. Serial dilutions were conducted, and plaque assays were performed to enumerate MS2.

Results: MS2 reductions occurred with only well water and at each concentration of PAA. Well water resulted in a 2.69 ± 0.07 log PFU/tomato reduction, 20 ppm PAA resulted in a 4.95 ± 0.08 log

PFU/tomato reduction, 60 ppm PAA resulted in a 5.85 ± 0.03 log PFU/tomato reduction, and 80 ppm PAA resulted in a 3.71 ± 0.26 log PFU/tomato reduction.

Significance: Greater concentrations of PAA lead to higher reduction of viral surrogates, up to 60 ppm PAA, suggesting its ability to also control viral foodborne pathogens during produce washing in an overhead spray and brush roller system.

19. SALMONELLA ENTERICA SEROVAR INFANTIS CARRYING PESI-LIKE MEGAPLASMID ISOLATED FROM A CAT

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Introduction: Salmonella Infantis has become an increasingly predominant Salmonella serotype in broiler chickens, and human infections in the United States. Some S. Infantis isolates harbor a megaplasmid called pESI (plasmid of emerging S. enterica Infantis). Most pESI carry multiple resistance genes, including different variants of blaCTX-M, which encode ESBL. Specifically,

the emergence of multi-drug resistant (MDR) extended-spectrum beta-lactamase (ESBL)-producing S. Infantis in humans, food animals, and retail chickens underscores a significant public health concern.

Methods: Nontyphoidal Salmonella enterica serotypes isolated from clinical specimens submitted to the Bronson Animal Disease Diagnostic Laboratory (BADDL) in Florida from 2018 to 2023 were selected to identify emerging antimicrobial resistance (AMR) determinants. A polymerase chain reaction (PCR) was performed to detect the presence of pESI in the Salmonella isolates. Here, we identified the presence of pESI in the S. Infantis strain from the lungs of an adult male cat (12-year-old, domestic shorthair) with a history of hepatic cancer and subsequently succumbed to pneumonia. Whole genome sequencing was performed using Illumina and Oxford Nanopore technologies. The genetic characteristics of the pESI were analyzed using CLC Genomics Workbench, Rapid Annotation using Subsystems Technology (RAST), Comprehensive Antibiotic Resistance Database (CARD), ResFinder, and Proksee.

Results: This S. Infantis isolate harbored a pESI-like megaplasmid, 255,759 bp, and contains genes conferring multi-drug resistance and virulence.

Significance: To our knowledge, this is the first report of S. Infantis harboring the pESI-like megaplasmid isolated from a cat. This finding highlights the significant potential risks of pet-associated MDR S. Infantis infections in household settings to humans.

20. SALMONELLA-INFECTED MACROPHAGE-DERIVED EXTRACELLULAR VESICLES STIMULATE A NOVEL SUBSET OF TYPE-2 CONVECTIONAL DENDRITIC CELLS TO SUPPORT PROTECTIVE IMMUNOMODULATORY RESPONSES.

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Salmonellosis is ranked the second zoonic foodborne disease of concern that is able to infect a vast range of animal and human hosts. The compounded health and agricultural detrimental consequences of Salmonella along the rapidly evolving antibiotic resistance strains has propped the need to develop novel therapeutic venues to prevent and treat these and potential infectious threats. Host-derived extracellular vesicles are critical components of cell-to-cell communication, which have been regarded for the ability to induce immunomodulatory effects during Salmonella enterica serovar Typhimurium (S. Tm) infection. Susceptible mice (BALB/c) challenged with oral S. Tm challenge recapitulate a model of typhoid fever inducing gastroenteritis, host systemic damage, and host death. Preliminary findings from our group indicate small extracellular vesicles (sEVs) derived from S. Tm-infected macrophages prime both innate and adaptive arms of the immune system. Antigen presenting cells encompass dendritic and macrophage cell immune cells, which migration and activation are essential for the interactions with T cells for shaping adaptive immune responses against secondary

pathogenic exposure. Thus far, intranasal dose of sEVs has been associated with both heighten host innate defense inducing strong pro-inflammatory innate immune responses (Th1 and M1-biased), cross-reactive IgG and IgA antibody titers, and survival against lethal salmonellosis. However, the mechanistic link bridging innate to adaptive immunomodulatory effects offered by the protective phenotypes induced when these sEVs are dosed intranasally has not been explored. In this study, BALB/c mice are intranasally dosed with sEVs from mock or S.Tm-infected RAW 264.7 macrophages (S.Tm-MO sEVs). Following 24-hours, mouse lungs, and spleens were dissected for flow cytometry analyses of antigen presenting cells subpopulations. Our results indicate significant increase in lung infected-type-2 conventional dendritic cells (cDC2) and interstitial macrophages in lungs and spleens from mice dosed with S.Tm-MO sEVs compared to mock and vehicle groups. We further interrogated changes in various organs linked to protective immune responses and antigen presenting cell activation using gene expression assays. Similarly, differential gene expression of murine Cd301b and Ccr7 were significantly induced in peripheral tissues (lungs, small intestine, and spleen). In conclusion, our results indicate S.Tm-MO sEVs uniquely stimulate antigen presenting cellular immune responses to link protective innate and adaptive immune responses against murine lethal typhoid fever challenge.

21. SPECIES IDENTIFICATION AND BIOCHEMICAL PROFILING OF NOVEL GUT ISOLATES: COMPARATIVE INSIGHTS AGAINST CLOSTRIDIOIDES DIFFICILE FOR PROTECTION MODELS

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Clostridioides difficile is a gram-positive spore-forming pathogen that infects the intestinal lining, causing severe inflammation and diarrhea. Current treatments for C. difficile infections (CDI) such as antibiotics and fecal microbiota transplants, often fail to prevent relapses and may pose risks like fostering opportunistic pathogens, highlighting the need for new bacterial therapeutics. To address this, we colonized germ-free mice with human donor-derived Firmicutes (Bacillota) species, isolating six unique species for evaluation to identify key protective candidates. Identification of these isolates was conducted using the Bruker MALDI Biotyper Sirius assay and 16S rRNA gene sequencing with Nanopore MinION technology. Interestingly, the sequencing revealed multiple species sequence matches for individual isolates, indicating the presence of multiple 16S rRNA gene copies, a known fitness mechanism in the phylum Firmicutes. Biochemical characterization of the isolates was performed using API 20A assay kits, which included key sugar utilization tests, and additional biochemical assays such as nitrate reduction, lipid, casein, and

starch hydrolysis, motility, hemolysis, and sulfide production were performed. Comparative analysis with published data for closely related species provided insights into the metabolic and functional capabilities of the isolates. Recent findings showed that two of the isolates, Paraclostridium 691 and Hungatella 659, were able to synergistically confer asymptomatic protection against CDI in vivo. Previous research indicates that nutritional competition is a key mechanism in restricting C. difficile growth. The metabolism of sugars, particularly trehalose, is known to correlate with the increased virulence and fitness of C. difficile. Our results highlight Hungatella 659's utilization of trehalose, suggesting direct competition with C. difficile for this vital nutrient, potentially limiting the pathogen's ability to thrive and cause disease. Upcoming studies will use PacBio WGS and ex vivo omics to explore the genetic and metabolic mechanisms behind these isolates' protective effects. This will reveal key genomic traits shaping gut microbial dynamics and offer new strategies to combat C. difficile. By characterizing protective Firmicutes, this research highlights the potential for targeted bacterial therapies to overcome current treatment limitations for CDI.

22. THE GUT HYPOXIC ENVIRONMENT FAVORS CRYTOSPORIDIUM PARVUM INFECTION OF HUMAN INTESTINAL EPITHALIAL CELLS

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Cryptosporidium parvum (C. parvum) is a eukaryotic single-celled enteric pathogen that causes intestinal inflammation and diarrhea. Importantly, infection can lead to potentially life-threatening disease in immunocompromised individuals. Around 130,000 people die each year from C. parvum infection, and it is the fifth leading cause of diarrheal death in children under five. C. parvum is ingested through contaminated water and is activated in the intestines of the host, allowing the parasite to invade intestinal epithelial cells (IECs) lining the gastro-intestinal tract. The lumen of the gut is characterized by low concentrations of oxygen referred to as hypoxia. Hypoxia is critical for the maintenance of a healthy microbiota and gut homeostasis. However, the impact of low oxygen concentrations on C. parvum infection of IECs remains unknown. Previous work performed in our laboratories has revealed that hypoxia favors enteric virus infection, which led to the hypothesis that hypoxia might also favor C. parvum infection.

Using intestinal epithelial cell lines and primary intestinal organoids, we have discovered that C. parvum replication is

significantly increased under hypoxia. It was previously shown that C. parvum siphons glucose and glucose-6-phosphate (G6P) from the host cell and relies on glycolysis to provide energy for replication. Under hypoxia, IECs shift their metabolism toward glycolysis through increased expression of glucose transporters (GLUT1) and glycolytic enzymes, since oxidative phosphorylation can no longer take place. Thus, we investigated whether the hypoxia-induced host cell upregulation of glycolysis and glucose uptake promotes C. parvum infection.

Manipulation of cellular glucose uptake through the use of GLUT1 inhibitors, genetic modification of GLUT1 and varying glucose concentrations in media, showed that the increased replication of C. parvum under hypoxia relies on increased host cell glucose uptake. Additionally, we found that not only increased glucose concentration, but also an increase in its conversion to glucose-6-phosphate, is critical for the hypoxia-mediated increase of C. parvum replication. This highlights how hypoxia-induced metabolic shifts in the host that favor glycolysis create an environment that promotes C. parvum infection. As our next step, we are performing RNA sequencing to gain a broader understanding of how hypoxia impacts not only the host, but also how it impacts the parasite itself to ultimately favor parasite replication. This project will provide novel insights into how the hypoxic gut environment contributes to C. parvum infection and replication. A better understanding of these processes will offer potential novel therapeutic targets to combat C. parvum infections.

23. THE IMPACT OF DIFFERING LIPID PROFILES OF BACTERIAL EXTRACELLULAR VESICLES ON INDUCTION OF ANTIVIRAL IMMUNE RESPONSES AND MURINE NOROVIRUS INFECTION

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Commensal bacteria regulate infection of every enteric viral pathogen investigated to date, however the mechanisms behind this regulation are still unclear for several viruses. During murine norovirus (MNV) infection, little to no disruption of the epithelial barrier occurs, and commensal bacteria remain confined to the intestinal lumen. This indicates that a bacterial product, not the bacterium itself, is likely the regulator of infection. We have found that MNV infection increases the production of bacterial extracellular vesicles (bEVs) by commensal bacteria and that these vesicles lead to increased expression of inflammatory immune responses during bEV-MNV co-infection. Inflammatory responses induced by bEVs are associated with vesicle content, and we have shown that incubation with MNV alters the protein, lipid, and DNA content of bEVs from commensal bacteria. Lipidomic analysis of bEVs shows that incubation with MNV resulted in significantly different lipid profiles from those produced in the absence of virus, and that virally induced bEVs were more abundant in

glycerophospholipids and sphingolipids. Both lipid classes are known stimulators of inflammatory immune responses and are associated with control of viral infection. Then, the role of bEV associated sphingolipids in inducing antiviral immune responses and viral infection was investigated. RAW 264.7 macrophages were treated with bEVs produced from wild-type and sphingolipid knockout bacterial strains and gene expression was evaluated using RNAseq and RT-qPCR. The impact of sphingolipids on MNV-bacterial binding and MNV infection of macrophages was also investigated. Experiments on the effect of bEVs on MNV replication in RAW cells were also repeated in TLR4 KO cells. Results revealed altered gene expression profiles between cells treated with the two bEV types, with differential expression of several inflammatory pathways observed. Differences in viral attachment and replication were also observed. Understanding the molecular pathways induced by bEVs to regulate antiviral responses may provide a mechanistic framework for the identification of targets to prevent or treat norovirus infection.

24. UNCOVERING MICROBIAL INTERACTIONS THAT CONFER ASYMPTOMATIC PROTECTION AGAINST CLOSTRIDIOIDES DIFFICILE INFECTION

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Clostridioides difficile is a leading cause of nosocomial diarrhea in the U.S., affecting over 500,000 people annually and causing around 30,000 deaths. Current therapies, such as antibiotics, often fail to prevent recurrent infections, while fecal microbiota transplants (FMT) carry risks of introducing potential pathogens. To address these limitations, we are developing a mouse model to investigate how beneficial gut bacteria provide asymptomatic protection against CDI. Members of the phylum Firmicutes are known to provide protective effects against C. difficile. To identify key species involved in this protection, we colonized germ-free (GF) mice with human donor-derived gut Firmicutes and isolated two mouse-compatible novel strains, Paraclostridium strain 691 and Hungatella strain 659, from their fecal samples. These strains were tested for protective effects against CDI in a mouse infection model, leveraging GF mice's sterile gut and high vulnerability to CDI for precise study of microbial interactions. Mono-colonization experiments revealed that GF mice colonized with Paraclostridium

691 survived infection but exhibited clinical symptoms (weight loss and diarrhea), whereas GF mice colonized with Hungatella 659 became moribund within 36 hours post-infection. Interestingly, dual colonization with Paraclostridium 691 and Hungatella 659 provided complete asymptomatic protection. GF mice co-colonized with both strains exhibited a significantly lower C. difficile burden at 36 hours post-infection compared to the mono-colonized groups. Notably, these mice also had reduced vegetative C. difficile, the replicative, toxin-producing state, highlighting the inhibition of C. difficile growth and toxin production. These findings indicate that Paraclostridium 691 provides baseline protection essential for survival, while Hungatella 659 enhances this effect by promoting asymptomatic protection. Together, they synergistically inhibit C. difficile and reduce disease symptoms. Ongoing work focuses on uncovering microbial mechanisms and pathways driving this protection. Insights gained from this model could inform the development of targeted bacterial therapeutics, potentially a safer and more effective alternative to existing treatments, addressing the growing challenges of CDI.

25. VIRULENCE AND ANTIMICROBIAL RESISTANCE GENE PROFILES OF SALMONELLA ISOLATED FROM CAPTIVE REPTILES

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Introduction: Non-typhoidal Salmonella is responsible for 1.2 million cases of illness and 450 deaths annually in the United States. Though human salmonellosis is known as a foodborne illness, contact with animals may also be a significant source of infection. Many cases of reptile-associated salmonellosis are undiagnosed, leading to an underestimation of the true public health burden. The emergence of Antimicrobial Resistance (AMR) and Multidrug Resistance (MDR) in Salmonella has further complicated treatment and control measures. Therefore, this study aims to examine the AMR and virulence factor (VF) genes in Salmonella isolated from gastrointestinal tracts (GIT) and systemic (SYS) infections of captive reptiles in Florida from 2018 to 2023.

Methods: Whole genome sequence (WGS) data of 7 GIT and 3 SYS Salmonella enterica isolated from captive turtles, snakes, tortoises, and alligator at the State Bronson Animal Disease Diagnostic

Laboratory of Florida were analyzed using the Bacterial and Viral Resource Centre information system. WGS data were assembled using Unicycler and annotated using the RAST toolkit. Genes homologous to AMR and VF were identified using PATRICK and VFDB databases, respectively. Heat maps and graphs were generated using Python with Seaborn and Matplotlib libraries. Microsoft® Excel® was used for data filtering and tabling.

Results: Nine isolates were identified as Salmonella enterica subspecies enterica, while one SYS isolate belonged to Salmonella enterica subspecies diarizonae. Phenotypic antimicrobial susceptibility test identified aminoglycoside (35%) cephalosporins (35%), gentamycin B & C (25%), and tetracycline (5%%) resistance in the study isolates. Seventy percent isolates showed MDR. Genomic AMR analysis confirmed phenotypic results. Further, WGS revealed that the highest number of AMR genes were associated with multidrug efflux systems against fluoroquinolones, quinolones, phenoms, tetracyclines and cephalosporins, the commonly used antibiotics in captive reptiles. A total of 123 VF genes were common in Salmonella isolated from both infection types, while 23 and 17 genes were unique for GIT and SYS infections, respectively. Most of the VF genes are classified into Type III secretory system, invasion, and immune evasion in both GIT and SYS isolates. More host immune evasion genes were found in SYS isolates than in GIT isolates.

Conclusion: This study identified AMR and VF genes in Salmonella of reptile origin, which may enhance the ability of Salmonella to pose a risk to humans, particularly those in close contact with reptiles. It underscores the importance of public health programs to focus on mitigating the risk of reptile-associated salmonellosis and possible transfer of AMR to humans.

26. ANTIVIRAL ROLE OF LACTOBACILLUS ACIDOPHILUS SURFACE LAYER PROTEIN-A

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Seasonal Influenza causes millions of hospitalizations and thousands of deaths in the USA. The conventional preventive measure for influenza A is seasonal vaccination, which has varied protection in diverse groups of individuals from 40-75%. Antivirals are used in influenza cases diagnosed early. Otherwise, symptomatic treatment has been employed primarily. However excessive neutrophil inflammation is associated with acute lung injury during infection. Therefore, therapeutic strategies targeting hyperactive neutrophils may be useful for mitigating influenza-A induced cytokine derived hyperinflammation in the lungs. The gut-lung microbial connection implicates the microbiome as a new strategy to reduce disease severity and increase host immunity. Here, we propose a component derived from gut microbiota that can prevent Influenza disease by boosting the host's immunity. The surface layer protein A (SlpA) of Lactobacillus acidophilus expressing Lactococcus lactis (R110) has been a proven immunomodulator in the gut under human clinical trials. We found that oral feeding of R110 prevents neutrophils influx, a key mediator for lung inflammation. Additionally, we found that IL27 pathway is necessary for regulating SlpA-mediated anti-

inflammatory responses. Further studies are in progress to develop a mechanistic understanding of these events.

27. BIRD FLU IS THE NEW TREND: A QUALITATIVE CONTENT ANALYSIS OF HPAI SOCIAL MEDIA MESSAGING

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Introduction: Highly Pathogenic Avian Influenza (HPAI), also known as Bird Flu, is a type A virus that primarily affects birds but can also impact poultry, cattle, and other mammals. The USDA, working with State officials and other governmental agencies, has been responding to HPAI in U.S. poultry and dairy since February 2022, which has increased public health efforts. In a post-pandemic world, social media platforms such as TikTok are powerful tools to provide effective science communication.This study investigates science communication videos on TikTok regarding the most recent HPAI outbreak.

Method: This study used the social amplification of risk (SAoR), which posits there is an intersection between psychological, social, cultural, and institutional factors that intensify participants' perception of risks, as a guiding theoretical framework. A qualitative content analysis was performed to determine who was delivering messages about HPAI, the types of accounts videos were posted, and emergent themes. There were 66 videos posted

between 2/1/23-7/31/24 found with the following hashtags “HPAI”, “BirdFlu”, or “H5N1”. The Constant Comparative Method (CCM) was used to analyze data through a process where moments in each video were compared to subsequent videos, allowing for patterns to emerge.

Results: Major themes that emerged were: Public Health, Disease Detection, Relevant Occupations, Effected Groups, Response, Disease Conditions, Interventions, Wildlife, Communication, Bird Flu, Domesticated Animals, and Agricultural Products. Video length varied from five to 229-seconds, with the majority being 60-seconds or less. Themes related to videos included: poking fun at the topic, news segments, general information, and assessing concerns. Video creators included content-specific scientists (epidemiologists, microbiologists, veterinarians, physicians), news outlets, and concerned citizens. Health organizations like the CDC were not represented. Those with scientific-public health backgrounds focused primarily on health effects while concerned citizens focused on how the outbreak would impact pets and family members.

Conclusions: The onset of the HPAI outbreak has emphasized the need for timely, effective science communication strategies, especially during times of uncertainty. This applies to traditional media and social media platforms. During times of crisis, official channels are used to communicate to the public; however, social media strategies are an important part of the evolving conversation surrounding science communication and can amplify the risk. Further research should be conducted on how scientists and practitioners can use social media platforms, such as TikTok, to expand the reach of science communication messages. These efforts help establish a collaborative approach between different stakeholders for proactive risk communication strategies.

28. COMMUNICATING SCIENCE: TRUST IN THE TIME OF AVIAN INFLUENZA(H5N1) OUTBREAKS

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Outbreaks of zoonotic diseases have become more frequent and widespread (Gebreyes et al., 2020). These disease outbreaks can destabilize food systems, resulting in food insecurity and various downstream impacts (Patterson et al., 2020). Several cases of Avian Influenza (H5N1) associated with dairy and poultry workers, including one death, have been reported in the United States (CDC, 2025). It is important to maintain trust in the U.S. agricultural food system, as a lack of trust contributes to misconceptions and negative perceptions about the system (Goodwin et al., 2011; Settle et al., 2017). This study utilized a quantitative survey methodology to determine the variables that influence trust in science and the agricultural food system during an Avian Influenza outbreak. The health belief model (HBM) was employed to explain the factors influencing trust in science and trust in the agricultural food system during a zoonotic disease crisis. Results indicated that the science communication intervention had significantly increased scores of trust in science and the agricultural food system ($p < .001$). A paired sample t-test revealed differences in trust in science and trust in the agricultural food system after the science communication intervention. Also, a multiple linear regression

indicated a non-significant relationship between trust in science, risk perception ($\beta = -0.117$, $p = 0.069$, $n = 258$), and self-efficacy ($\beta = -0.046$, $p = 0.477$, $n = 258$). A significant relationship existed between perception of the agricultural food system and trust in the agricultural food system ($\beta = -0.148$, $p = 0.02$, $n = 258$). Similarly, the result revealed that participant self-efficacy positively influenced their trust in the agricultural food system ($\beta = .170$, $p = 0.006$, $n = 258$). Results confirm that HBM principles, specifically, perception of the agricultural food system, self-efficacy, and cue to action (science communication) significantly impacted trust in the U.S. agricultural food system. Science communication also impacted trust in science, however, there was no notable relationship between HBM principles, specifically, risk perception, and self-efficacy on trust in science. The findings of this study suggest that science communication can be used as a tool to improve general science literacy and enhance public confidence and self-efficacy during disease outbreaks. It is recommended that science communication interventions be adopted as an effective strategy to mitigate the potential impact of future disease threats. Future research should replicate this study with a general audience to enhance generalizability.

29. COMPARISON OF REAL-TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION AND DIGITAL DROPLET POLYMERASE CHAIN REACTION FOR THE DETECTION OF INFLUENZA A VIRUS IN WASTEWATER AND MILK SAMPLES

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Background: Highly pathogenic avian influenza (HPAI) H5N1 virus has caused significant outbreaks in wild birds, poultry, dairy cattle, small mammals, and humans. Reliable and timely detection methods are important for surveillance and control measures. The gold standard for HPAI H5N1 diagnostics is real-time reverse transcription polymerase chain reaction (qRT-PCR). However, low concentration samples (i.e. milk and wastewater) may not be identified if they are below the detection threshold of the assay. Emerging technologies like digital droplet PCR (ddPCR) may provide greater sensitivity of detection, increasing the utility of molecular diagnostic approaches for low concentration samples.

Methods: Wastewater and retail milk (pasteurized and unpasteurized) were collected from [March 2024–February 2025] in Florida. Samples underwent RNA extraction and were analyzed using qRT-PCR and dd-PCR for influenza A virus. Positive samples were also subtyped using the CDC HPAI H5N1 assay. Data from both assays were compared for their sensitivity and the influenza A virus detection threshold was calculated for both.

Results: Of the 155 wastewater samples collected and tested 45 (29.03%) were positive for influenza A virus by qRT-PCR. Of the 37 milk samples collected and tested 0 (0%) were positive for influenza A virus by qRT-PCR and 2 (5.4%) were positive by ddPCR. No samples tested positive for H5N1. Analysis of dilution curves for both assays showed ddPCR to have a slightly lower detection threshold (0.425 cp/ul) compared to qRT-PCR (15.3 cp/ul).

Conclusions: Influenza A virus was more prevalent in wastewater during the winter season. No samples were positive for H5N1 suggesting that samples may have contained human influenza A virus. The ddPCR detection method was more sensitive in detecting positives compared to qRT-PCR, suggesting that ddPCR may be a more optimal approach for testing low concentration samples.

30. INCORPORATING GENOMIC SEQUENCES INTO STOCHASTIC TRANSMISSION MODELING TO IMPROVE THE FORECASTING OF THE SPREAD OF SARS-COV-2

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Introduction: The recent SARS-CoV-2 pandemic has highlighted the growing importance of early-stage infectious disease forecasting. An accurate predictive model can empower public health leaders to make timely decisions on isolation and vaccination policies, thereby reducing the number of infected and severe cases. However, the emergence of new variants and subvariants can significantly alter the transmissibility and virulence of the pathogen in a short time, making the number of infections and hospitalizations difficult to predict. To enhance the timeliness and accuracy of forecasting, SARS-CoV-2 sequencing data can be utilized, which is a vast database as millions of sequences have been collected and reported over the past few years.

Methods and Results: We collected 10,000 complete SARS-CoV-2 sequences with high coverage and defined an index, the ”sequencing distance”, to measure the rate of evolution in the pathogen over time. We used partially observed Markov process together with stochastic compartmental model to study the transmission dynamics of SARS-CoV-2. By comparing the goodness of fit of the model on epidemiological data with and without considering the influence of genomic data on transmissibility, waning immunity and virulence of the infectious agent, we conclude that genomic data is crucial for capturing trends when

new variants and subvariants emerge, leading to the development of a more reliable forecasting model.

31. ON-THE-SPOT DETECTION OF AIRBORNE VIRUSES ENABLED BY A 3-D PRINTED INTERFACE BETWEEN AN AEROSOL COLLECTOR AND A MICROFLUIDIC DEVICE

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The need for detection of airborne viruses is crucial to prevent the spread of infectious diseases, as shown in the most recent pandemic caused severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has highlighted a need for testing methods that allow a faster turnaround from sample collection to testing result enabling appropriate responses. This paper reports on a low-cost 3D printed microscale interface that enables integration of a collector with a microfluidic detector for on-the-spot detection which utilizes a condensation-based method for collection of aerosolized airborne viruses.

We have developed a viable virus aerosol sampler (VIVAS) that has been shown to collect nanoscale virus particles through the use of condensation-based air sampler. VIVAS typically collects the virus particles in the air into a petri dish from which the contents can be extracted and cultured as live viruses. However, by collecting directly into a virus lysis buffer within an integrated adapter, we are able to collect a virus lysate. To perform nucleic acid tests, the sample must be purified and any agents that may inhibit detection assays removed. Valve-enabled lysis, paper-based RNA enrichment, RNA amplification device, VLEAD, performs this necessary step by collecting and enriching target nucleic acid onto a paper substrate in a singular device using valves to control the release of buffers enabling immediate on-the-spot testing.

Previous work has shown VLEAD can detect both SARS- CoV-2 and 2 influenza viruses. To demonstrate the integration of the VIVAS and VLEAD, this work integrates a nucleic acid amplification-based testing system directly into an aerosol-based collection mechanism using human coronavirus OC43 (HCoV-OC43). This system facilitates the ability to perform on-the-spot testing for a HCoV-OC43 and other aerosol viruses. Potential concerns about samples losing some amount of virus from residing within the collector were addressed by comparing the limit of detection of virus directly deposited into the VLEAD and samples collected through VIVAS using the adapter. The results of this work show no loss in sensitivity when using the adapter for sample collection indicating its viability for virus collection. Additionally, samples were able to be collected and evaluated with results appearing approximately an hour after finishing collection. This work demonstrates the viability of collection for on-the-spot detection using a microfluidic device.

32. ORGANIZATIONAL FINANCIAL DETERMINANTS OF COVID-19 NON-PHARMACEUTICAL INTERVENTIONS IN U.S. PUBLIC UNIVERSITIES

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Introduction: Non-pharmaceutical interventions (NPIs) such as physical distancing, capacity limits, and surveillance testing were essential tools for mitigating the spread of SARS-CoV-2. Public universities, however, faced complex decisions: balancing public health goals against financial realities. As semi-autonomous institutions, their choices affected campus health and contributed to the broader effectiveness of community pandemic responses. Yet, little research has examined how financial health influenced institutional NPI adoption. Beyond epidemiology, public health practice must improve its ability to engage institutional partners in conversations about the economic implications of interventions. Studies have shown that people from differing ideological perspectives often converge on similar preferences when discussing NPIs in terms of shared trade-offs, weighing infection risks, financial strain, and impacts on family stability. This highlights the power of framing where public health can strengthen alignment across diverse stakeholders by positioning NPIs as safety tools and as part of broader economic and institutional resilience strategies.

Methods: We conducted a cross-sectional analysis of a stratified random sample of U.S. public universities, assessing five NPIs implemented during Spring 2021 campus reopening: return to face-to-face instruction, dining facility opening, surveillance testing, and classroom capacity limits. Logistic regression models evaluated associations between institutional financial indicators (e.g., endowment size, operating margins, auxiliary revenue) and NPI implementation. We are applying a difference-in-differences approach using Spring 2022 financial data to explore potential causal effects that compare financial outcomes before and after the pandemic across institutions with varying NPI adoption decisions.

Results: Preliminary findings indicate that financial characteristics were strongly associated with NPI choices. Universities that reopened dining facilities tended to have rising enrollment, more international students, cash-positive operations, and stronger auxiliary revenue streams. Institutions that maintained dining restrictions were associated with higher grant and contract income proportions. Surveillance testing was more common among large universities (20,000+ students), those with affiliated hospitals, and those reporting strong enrollment trends and higher gross revenue.

Conclusion: Organizational financial health plays a role in shaping pandemic response decisions. Public health efforts that ignore this dimension risk misalignment with institutional partners. Integrating financial literacy and framing strategies into public health preparedness can foster more effective collaboration and support NPI implementation that is both epidemiologically sound and operationally feasible.

33. WILDLIFE REHABILITATION FACILITIES, ESSENTIAL PARTNERS IN ONE HEALTH COLLABORATIVE NETWORKS: SARS-COV-2 SURVEILLANCE IN NATIVE U.S. MAMMALS AND ASSESSMENT OF BIOSECURITY PRACTICES

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Introduction: Wildlife rehabilitation groups are overlooked partners in research and often find themselves on the front lines of endemic, emerging, and reemerging diseases of one health importance. Considerable human-animal interactions occur at wildlife rehabilitation facilities, where ill or injured wildlife are temporarily housed in human care. Animals infected with SARS-CoV-2 can potentially infect other animals and humans in close contact. This study further investigated susceptibility of US native wild mammals to SARS-CoV-2 infection and surveyed biosecurity practices utilized by wildlife rehabilitation facilities for reducing potential transmission. This effort required a collaborative team involving USDA-APHIS, TGen, UF-Emerging Pathogens Institute (UF EPI) and a network of over 69 wildlife rehabilitators across 39 states.

Methods: The UF-EPI team acquired state permissions or permits and recruited U.S. facilities who collected respiratory swabs, rectal or fecal swabs, and blood samples from arboreal, terrestrial, or aquatic non-endangered native mammals. Samples were submitted to TGen with swabs screened by RT-qPCR (viral detection) and serum by viral neutralization assays (antibody detection). Biosecurity surveys were completed by facilities to identify and formulate best practice guidelines for disease prevention at this human-wildlife interface.

Results: Since 2023, more than 2400 swab samples and 950 blood samples from 62 species have been screened for virus and antibodies against SARS-CoV-2, respectively. While the project is ongoing, so far virus was detected by RT-PCR in an eastern gray squirrel, a bobcat, and an opossum, while neutralizing antibodies against SARS-CoV-2 were detected in 10 white-tailed deer fawns, a bobcat, and a beaver.

Conclusions: This study expands our understanding of species susceptibility, provides insight on biosecurity practices protecting both humans/animals, and illustrates the importance of establishing One Health collaborative networks with the wildlife community for current and future research and disease surveillance.

34. A GNOTOBIOTIC APPROACH TO ELUCIDATING RICE BLAST-RICE MICROBIOME INTERACTIONS

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The microbiome of eukaryotic species influences a wide range of host systems, significantly affecting host fitness. In plants, the interplay between microbiome stability and host immune function plays a critical role in developing immunocompetency. The model pathosystem involving the fungal pathogen Magnaporthe oryzae (the causative agent of rice blast) and Oryza sativa (rice) offers a valuable opportunity to study this interaction given the extensive knowledge available on the system and its critical role in global food security. Due to the hemibiotrophic nature and genomic plasticity exhibited by M. oryzae, traditional mitigation strategies have not conferred reliable control of the disease, and thus alternative mitigation strategies are required. Rice blast destroys enough rice annually to feed approximately 60 million people, thus creating a large economic and societal impact. Using this model pathosystem, we investigate the interactions between the rice microbiome and the fungal pathogen M.oryzae through the use of a gnotobiotic growth system. By conducting spray infection assays on selectively colonized and germ-free plants, we have characterized disease severity and host transcriptional state in each treatment. In alignment with results from gnotobiotic experiments conducted in Arabidopsis, germ-free plants show an increase in disease severity and show signs of autoimmunity represented by significantly upregulated defense response genes in the absence of a pathogen. This study sheds light on the impact of

microbiome dynamics during rice blast infection, uncovering novel mechanisms of disease resistance or susceptibility associated with microbiome composition.

35. A NON-CANONICAL ACTIVATION OF THE HOST'S ESCRT MACHINERY IS REQUIRED FOR THE SCISSION OF PARASITOPHOROUS VACUOLES AND THE REPLICATION OF LEISHMANIA DONOVANI

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Leishmania donovani (Ld) are the causative agents of visceral leishmaniasis. In mammalian cells, Ld live in vacuolar compartments called parasitophorous vacuoles (LdLPVs) that enigmatically divide following parasite replication. We evaluated the role of the endosomal sorting complex required for transport (ESCRT) machinery in the scission of LdLPVs. We found that ESCRT components are constitutively recruited to LdLPVs. This recruitment depends on the expression of PI(3,4)P2 on LdLPVs. The knockdown (KD) of upstream components of the ESCRT machinery revealed that KD of ESCRT accessory ALIX but not ESCRT-I TSG101 or VPS28 led to a significant reduction in the parasite burden in infected cultures. Interestingly, LdLPVs in ALIXKDs were more distended and harbored more than 2 parasites. Incorporation of BrdU into Leishmania in THP-1 macrophages revealed that parasite replication was inhibited in ALIXKD due to defective LdLPV scission. These findings establish that non-canonical activation of the ESCRT machinery is required for Leishmania to replicate within macrophages.

36. A WORK OF ART: CHARACTERIZING SECRETED PROTEIN ART12 AS A PUTATIVE EFFECTOR IN RICE BLAST PATHOGENESIS

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Rice blast is one of the most devastating agricultural diseases worldwide, caused by the hemibiotrophic fungus Magnaporthe oryzae. M. oryzae secretes effector proteins, enabling pathogenesis through immune suppression and subsequent necrosis of the plant host. As efforts to characterize the virulence mechanisms of this pathogen continue, of particular interest is a putative effector, which we refer to as ART12, that is structurally similar to an ADP-Ribosyltransferase belonging to the heat labile-enterotoxin cholera toxin. In a continued effort to characterize ART12, we investigated its secretion, localization, and expression during M. oryzae infection, and performed genetic manipulation to determine its role in the whole pathogenicity of the fungus. Through generation of a fluorescently tagged ART12 fusion protein, we observed ART12 to be secreted through the pathogen-specific Biotrophic Interfacial Complex (BIC), localizing to the plant host cytoplasm. Furthermore, we detected secretion of this effector beginning from 16 to 48 hours post-infection, which we then validated with qPCR analysis, indicating ART12 is likely involved in M. oryzae's biotrophic lifestyle, and ability to suppress host immunity. We also generated through CRISPR-Cas9 an ART12 knockout mutant, with which we performed infection assays. These assays showed a significant decrease in disease incidence, revealing ART12 is critical for full rice blast pathogenicity. These findings serve as the basis for the direction of future investigations that will continue to characterize and delineate the functional mechanisms of ART12,

giving insight into new roles for these effectors and their influence in the context of rice blast.

37. ANALYZING RISK FACTORS ASSOCIATED WITH EMERGING PATHOGEN CANDIDA AURIS IN A LARGE, TERTIARY CARE HOSPITAL: A CASE-CONTROL STUDY

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Background: Recently, Candida auris has emerged as a significant concern in healthcare settings due to its resistance to multiple drugs, its potential for being mistakenly identified as other Candida species, and its potential to be underreported. Previous studies described that C. auris can cause bloodstream infections and mortality to those who are immunocompromised, have invasive medical devices, or have longer hospital stays (Du et al., 2020; Hu et al., 2021).

Objectives: The study aimed to examine the association between previous facility types, presence of invasive medical devices, and time-to-conversion with testing positive for C. auris.

Methods: The retrospective case-control study examined patients admitted to UF Health Shands Hospital during January 3, 2023, to January 2, 2024. A sample size of 573 screened patients was determined, where 114 C. auris positive patients were classified as cases and 459 C. auris negative patients were controls. Multiple logistic regression analysis was done to determine the relationship between exposures of interest with testing positive for C. auris.

Results: Previous hospital (OR=9.916), long term acute care hospital (LTACH) (OR=7.735), and other stays defined as group homes, home hospice care, or senior living facilities (OR= 2.416) were risk factors associated with testing positive for C. auris.

However, only hospital (p-value=0.0001, 95% CI 3.103-31.689) and LTACH stays (p-value=0.0002, 95% CI 2.627-22.774) were statistically significant findings. The presence of abdominal feeding tubes (gastrostomy, jejunostomy, or percutaneous endoscopic gastrostomy tubes) were a risk factor associated with testing positive for C. auris (OR=2.373) as well as endotracheal tubes (OR=1.5). Abdominal feeding tubes were the only statistically significant findings amongst the invasive medical devices examined (p-value=0.0067, 95% CI 1.271-4.429). The median days between admission and testing positive for C. auris was 13.5 days.

Conclusions: The findings of this study reinforce the importance of targeting specific patient populations when screening for C. auris upon admission since they may be associated with testing positive for the pathogen. Future studies need to be conducted to gain deeper understandings of risk factors associated with C. auris to minimize the spread in hospitals.

38. ANTI-CRYPTOCOCCUS NEOFORMANS ACTIVITY OF ACINETOBACTER LWOFFII POTENTIALLY LINKED TO ITURIN A PRODUCTION

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Introduction: Cryptococcus neoformans is an encapsulated pathogenic yeast capable of causing meningoencephalitis mainly in immunocompromised individuals, in particular those with HIV/AIDS. C. neoformans is ubiquitously found in pigeon droppings which are found in diverse microbial interactions, such as with the bacteria Acinetobacter lwoffii. Our group has found by in silico and in vitro evidence that A. lowffii inhibits the growth of C. neoformans. Direct contact with A. lowffi was not necessary, since supernatants of single cultures of A. lowffi have also a detrimental effect on C. neoformans. Due to the present limitations of current antifungals such as Amphotericin B and Fluconazole, the study of possible alternative antifungals is crucial to curb the rise of resistance and toxicity of current therapeutic agents. Iturin A is a cyclic lipopeptide produced by Bacillus subtillis and Acinetobacter baumanii, known for its antifungal properties. Given its close relationship to A. baumanii, we hypothesized that Iturin A would be produced by A. lwoffi. Hence, our goal was to determine the production of Iturin A by A. lwoffii and elucidate its antifungal potential against C. neoformans.

Method: Experiments were done with Iturin A from Bacillus subtilis, C. neoformans strain H99 and A. lwoffi strain 1642. Supernatants were fractioned using Amicon filters of 3KDa. Minimal inhibitory concentration (MIC) determinations were done

according to the microplate method of Clinical and Laboratory Standards Institute. The cell membrane integrity assay was done by propidium iodide staining using C. neoformans untreated as control.

Results: Fractioned supernatants of A. lwoffii cultures of <3kDa showed higher percentages of C. neoformans inhibition compared with the other fraction (53% vs 36%). The MIC of Iturin A on C. neoformans was 12.5 mg/mL and it showed a synergistic effect with Amphotericin B 0.25 mg/mL where the MIC was reduced to 2.5 mg/mL, highlighting its potential use in antifungal treatments. Treatments with Iturin A induce cell membrane disruption in C. neoformans being significant at 4 h with 24% higher permeabilization than the untreated control.

Conclusions: Further studies will focus on confirming the production of Iturin A by A. lwoffii using mass spectrometry of supernatants and will also determine the effect of Iturin A on C. neoformans virulence factors, such as biofilm and capsule production. By confirming the antifungal properties of Iturin A, this work may pave the way for future research into its clinical applications, helping to address the growing threat of fungal infections in immunocompromised populations.

39. DEVELOPMENT OF NOVEL FUNGICIDES TO CONTROL NEOPESTALOTIOPSIS SP. (ASCOMYCOTA) INFECTIONS: AN EMERGING AND DESTRUCTIVE FUNGAL PATHOGEN IN STRAWBERRY PRODUCTION SYSTEMS

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Neopestalotiopsis sp. (Ascomycota: Sporocadaceae) is a filamentous Ascomycota that was identified in 2017 in unprecedented outbreaks in Florida strawberry fields, resulting in the loss of an estimated 80 ha of production fields over 18 commercial farms. Neopestalotiopsis sp. was found to have high aggressiveness and cause blight-like necrosis symptoms which consist in lesions on the strawberry fruit as well as the leaves, eventually killing the tissue despite the application of state recommended fungicides. As reported widely in the literature, in vitro toxicology studies in Ascomycota fungi are usually carried out on solid agar media, where fungal growth can take several days to weeks before recording a notable difference in growth. This prolonged growth timeline restricts the ability for high-throughput chemical screening approaches for the discovery of novel fungicides and thus, our goal is to develop methodology for fungal growth in liquid media that will allow fungicide testing on Neopestalotiopsis in a high-throughput manner. The first tests carried out in 96-well plates showed that Neopestalotiopsis sp. spores cultivated in Potato Dextrose Broth at 25°C can develop

hyphae in a distinguishable manner within 48 hours, thus making high-throughput testing possible. However, the hyphae growth occurred in a non-homogenous manner and further protocol optimization is underway to obtain reliable growth estimation through the measurement of optic density at 600 nm (OD600) in each well at regular intervals. A preliminary assay showed evidence that Neopestalotiopsis sp. growth was impaired by the presence of fludioxonil, which is a phenylpyrrole fungicide that inhibits fungal growth through disruption of phosphorylation of glucose. This has justified the testing of other natural phenylpyrrole compounds of synthetic and natural origins against Neopestalotiopsis sp. growth in laboratory and semi-field settings. The described pipeline for screening for fungicidal activity against Neopestalotiopsis sp. is critical for the development of novel chemistry and control of this emerging fungal pests. Data on methodology and proof-of-concept high-throughput screening will be presented.

40. ESSENTIAL ROLE OF HEPCIDIN IN HOST RESISTANCE TO DISSEMINATED CANDIDIASIS

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Background: Candida albicans (C. albicans) is a commensal fungus, and its transition from commensalism to pathogenicity remains the predominant cause of invasive candidiasis, with up to 40% mortality. While the risk of systemic candidiasis and clinical outcomes vary significantly, the host-related risk factors are not well-defined. Iron is an essential micronutrient for host physiology and a growth factor for invading microbes including C. albicans. Hence, iron metabolism is tightly regulated both at systemic and cellular levels. Systemic iron metabolism is primarily regulated by the hepcidin-ferroportin axis. Hepatic hormone hepcidin binds to and triggers the internalization of ferroportin, the only known mammalian iron transporter. Patients with chronic liver disease produce less hepcidin and are more susceptible to candidemia despite the absence of neutropenia. Whether impaired hepcidin production influences the outcomes of disseminating candidiasis has not yet been investigated.

Experiment: Genetic and tamoxifen-inducible hepcidin knockout (Hamp-/-) and their wild-type (WT) littermates were infected with

C. albicans SC5314. C. albicans was grown in iron-rich conditions and stained for beta-glucan. In separate experiments, synthetic hepcidin mimetic- PR73 was administered to a cohort of iHamp-/- mice.

Results: Genetic and inducible hepcidin deficiency resulted in rapid iron overload in the kidney and liver. However, the fungal burden in the kidney was orders of magnitude higher than in the liver. Compared to WT mice, genetic and inducible Hamp-/- mice displayed increased renal fungal burden, more renal injury, and inflammation. On day 3, C. albicans was in yeast form in the kidneys of WT mice but had transformed into hyphae in the iron-rich renal tubular segments of Hamp-/- mice. This was associated with loss in the renal parenchyma and increased mortality. PR-73 treatment reduced renal fungal burden, hyphal transformation, and renal injury in Hamp-/- mice. The fungus undergoes hyphal transformation in iron-rich in-vivo as well as in-vitro conditions while exposing beta-1,3-glucan, which can be recognized by the epithelial and immune cells eliciting a response.

Conclusion: Our data identify hepcidin deficiency as a novel host susceptibility factor in C. albicans-induced renal failure and mortality. Hepcidin deficiency was associated with increased renal iron burden and accelerated hyphal transformation, indicative of increased virulence. Since PR-73 mitigated C. albicans-induced pathology, our study identifies ferroportin as a druggable target to ameliorate outcomes of disseminating candidiasis. Our data lay the foundation for evaluating the efficacy of hepcidin analogs and drugs like Vamifeport (clinical stage oral ferroportin inhibitor) as possible therapeutic interventions.

41. EVALUATION OF SOIL BACTERIAL ISOLATES FOR THE BIOCONTROL AGAINST MAGNAPORTHE ORYZAE, THE CAUSAL AGENT OF RICE BLAST DISEASE

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Rice blast disease, caused by Magnaporthe oryzae, devastates 10-30% of global rice crops annually, posing a significant threat to food security. While chemical fungicides are commonly used for disease management, their effectiveness is often hindered by the rapid emergence of resistant fungal strains, environmental concerns, and potential health risks. This study explores biological control agents (BCAs) as a sustainable alternative to control for managing rice blast. We isolated several soil bacterial strains from various locations in the Gainesville area and identified three strains - DC01, DC09, and DC13 - that inhibited M. oryzae growth in dual-culture assays. To investigate their potential mechanisms, we

conducted volatile compound assays, revealing that all three strains inhibited fungal growth by 20-60% through the release of gaseous inhibitory compounds. Bacterial identification via 16S rRNA sequencing confirmed that all three strains belong to Bacillus pumilus. In addition to their antagonistic activity, we evaluated these strains for plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, nitrogen fixation, and drought tolerance. All strains produced IAA, a key phytohormone involved in plant growth and development, at concentrations ranging from 46 to 64 μg/mL within 48 hours of incubation. In phosphate solubilization assays, which evaluate a bacterium's ability to convert insoluble phosphate into bioavailable forms for plant uptake and soil nutrient cycling, only DC09 demonstrated the ability to utilize phosphate compared to positive control strains. Nitrogen fixation assays indicated that all three strains could utilize atmospheric nitrogen for growth under specific plate conditions. Additionally, these strains exhibited superior growth under PEG-simulated low, medium, and high drought conditions compared to control strains, with DC01 and DC13 displaying better drought tolerance than DC09, demonstrating variability among strains of the same species. Overall, in plate assays, DC09 exhibited the strongest potential, producing 64.02 μg/mL of IAA, fixing 62.10 μg/mL of nitrogen, and demonstrating robust phosphate solubilization. Furthermore, pre-treatment with these bacterial strains 24 hours before infection reduced necrotic lesion formation in rice plants, with gene expression analysis via qPCR revealing that these strains prime plant defense responses against M. oryzae infection. Collectively, our findings suggest that B. pumilus holds promise as an effective BCA for mitigating rice blast disease, offering a viable strategy for sustainable disease management.

42. FUNGAL FRONTIERS: REPURPOSING A NEUROTROPIC PATHOGEN TO SHUTTLE DRUG-LOADED NANOPARTICLES ACROSS THE BLOOD-BRAIN BARRIER

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Traditional drug delivery faces major challenges as pharmaceuticals are often cleared before reaching their targets. Many drugs also struggle to cross biological barriers like the blood-brain barrier, limiting treatment options for diseases such as Alzheimer's and Lou Gehrig's disease. To overcome such hurdles, innovative drug delivery strategies are critical. Cryptococcus neoformans (Cn), a fungal pathogen, naturally hijacks immune cells to disseminate throughout the body, including the brain. Upon arrival in the brain, Cn escapes from the confines of the immune cell without causing damage through "vomocytosis" or "non-lytic exocytosis". These unique properties make Cn an intriguing candidate for targeted drug delivery. We propose that an avirulent strain of Cn, functionalized with drug-loaded poly(lactic-co-glycolic) acid (PLGA) nanoparticles, could enhance drug accumulation in these tissues, improving therapeutic outcomes. This study focuses on two key aspects of fungal drug carriers (FDCs): surface engineering and biodistribution. We optimized nanoparticle attachment to Cn via intermolecular forces and bioconjugation while evaluating their impact on FDC viability,

particle stability, and vomocytosis. Using a bioluminescent Cn strain in mice, we assessed the effects of various delivery routes on trafficking efficiency to the brain. Finally, we compared the ability of FDCs to deliver nanoparticles to the brain against traditional nanoparticle administration. Our findings demonstrate a novel approach to overcoming physiological barriers, offering a promising strategy to enhance drug delivery to a generally inaccessible tissue and improve treatment for neurological diseases.

43. HEPCIDIN ENFORCES NUTRITIONAL IMMUNITY AGAINST SYSTEMIC CANDIDA AURIS INFECTION

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Introduction: The yeast Candida auris (C. auris) is designated as an urgent threat by the Centers for Disease Control and Prevention. Bloodstream C. auris infections in vulnerable patients are life-threatening and associated with high mortality. While C. auris is remarkably resistant to antifungals and evades immune recognition, host factors that increase the risk of infection are not defined.

Iron is an essential micronutrient for host physiology and a growth factor for invading microbes. Systemic iron metabolism is regulated primarily by the hepatic hormone, hepcidin, and its cellular receptor, ferroportin. Hepcidin-mediated ferroportin

degradation attenuates the iron entry rate into plasma and extracellular fluid, allowing transferrin to remove bioavailable non-transferrin-bound iron. Patients with chronic liver disease are more susceptible to candidemia. To address the biology underlying this critical clinical problem, we investigated the mechanistic link between hepcidin insufficiency and C. auris infection at immunological and microbiological level.

Methods: We assessed the growth of C. auris strains AR-0382 and AR-0386 in medium containing varying concentrations of iron. Immunocompetent C57BL/6 mice were injected with AR-0386 grown in standard medium or in an iron-rich medium. Female, immunocompetent hepcidin knockout and their wild-type (WT) littermates (on C57BLK/6 background) were infected with C. auris AR-0382 and AR-0382Δscf1 (lacking the ability to form biofilm, less virulent). The outcomes of infection were evaluated at different time points.

Results: Compared to standard medium AR-0382 and AR-0386 grew significantly faster in medium containing iron. C. auris grown in iron-rich medium formed clusters, exposed 1,3-b glucan, and elicited a significantly greater immune response from bone marrow-derived macrophages. WT mice infected with C. auris grown in an iron-rich medium died rapidly whereas those that received C. auris grown in a standard medium survived for 10 days. Compared to WT mice, Hamp-/- mice had significantly greater renal fungal burden, injury, and immune cell infiltration when infected with AR-0382 and AR-0382Δscf1 strains. Fungal burden was also significantly greater in other organs.

Conclusion: We demonstrate for the first time that C. auris utilizes iron for sustenance. In an immunocompetent host, we identify hepcidin deficiency as a susceptibility factor to C.auris infection. The control of immunity through hepcidin is specific to candida

species, as hepcidin deficiency does not influence the outcome of Aspergillus fumigatus infections. Our findings lay the foundation for evaluating the efficacy of hepcidin mimetics such as Vamifeport and Rusfertide, as adjunct therapy to control C. auris infections and improve the prognosis of at-risk patients.

44. IL-6 DEFICIENCY ACCELERATES CEREBRAL CRYPTOCOCCOSIS AND ALTERS GLIAL CELL RESPONSES

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Introduction: Cryptococcus neoformans (Cn) is an opportunistic encapsulated fungal pathogen that causes life-threatening meningoencephalitis in immunosuppressed individuals. Interleukin-6 (IL-6) is crucial for blood-brain barrier integrity, and its deficiency has been shown to facilitate Cn brain invasion. This study investigates the impact of IL-6 on systemic Cn infection in vivo, focusing on central nervous system (CNS) colonization and glial responses, specifically microglia and astrocytes.

Methods: We utilized IL-6 knock-out (IL-6−/−) mice, wild-type C57BL/6 (WT) mice, and IL-6−/− mice supplemented with recombinant IL-6 (rIL-6; 40 pg/g/day). Mice were infected intravenously with C. neoformans strain H99. Fungal burdens in the lungs, blood, and brain tissues were quantified at 7 days post-infection using colony-forming unit (CFU) assays. Histological analyses assessed pulmonary inflammation and CNS involvement.

For the in vitro assays, C. neoformans was exposed to rIL-6 to evaluate capsule growth. Primary microglia-like cells were cultured with rIL-6 to assess phagocytosis and fungal killing, while IL-6 silencing was performed to determine its role in microglial function. Astrogliosis was evaluated using immunohistochemical staining for glial fibrillary acidic protein (GFAP).

Results: IL-6−/− mice exhibited faster mortality compared to WT and IL-6−/− mice supplemented with rIL-6. Early lung inflammation was observed across all groups, with no major histological differences in pulmonary cryptococcosis progression. However, IL-6−/− mice had significantly higher fungal burdens in blood and brain tissues at 7 days post-infection. In vitro exposure of C. neoformans to rIL-6 increased capsule size, suggesting a direct effect of IL-6 on the fungus. IL-6−/− brains showed an increased number of dystrophic microglia during Cn infection, which are associated with neurodegeneration and senescence. In contrast, the brains of IL-6-producing or IL-6-supplemented mice displayed high numbers of activated and phagocytic microglia, indicating a stronger anti-cryptococcal response or tissue repair. Microglia-like cells cultured with rIL-6 exhibited enhanced fungal phagocytosis and killing, whereas IL-6 silencing in microglia decreased fungal phagocytosis. Astrogliosis was pronounced in infected brains from WT mice and moderate in IL-6−/− mice supplemented with rIL-6, while minimal astrogliosis was observed in IL-6−/− tissue, highlighting the potential of astrocytes in containing and combating cryptococcal infection.

Conclusions: Our findings suggest a critical role for IL-6 in Cn CNS dissemination, neurocryptococcosis development, and host defense. IL-6 deficiency exacerbates CNS fungal invasion by impairing microglial and astrocytic responses. Enhancing IL-6

signaling may represent a therapeutic strategy to strengthen host defenses against cryptococcal infections in vulnerable populations.

45. INVESTIGATING THE ROLE OF COAGULATION IN THE MALARIA-ASSOCIATED RESPIRATORY DISTRESS SYNDROME

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Malaria is a bloodborne parasitic disease transmitted by mosquitoes. Severe cases of malaria can develop into malaria-associated acute respiratory distress syndrome (MA-ARDs), marked by pulmonary complications such as edema, inflammation, and hemorrhages, and leading to lung damage, respiratory failure and death. While inflammation is associated with triggering a strong immune response, the role of coagulation in MA-ARDs and sex-based differences driving morbidity are poorly understood. To address this question, we established an in vivo murine model lacking the expression of tissue factor, the primary initiator of the blood coagulation cascade, in endothelial and haemopoietic cells. We hypothesized that altered tissue factor expression from endothelial and haemopoietic cells will impact MA-ARDS outcomes. Preliminary results reveal sex-based differences in MA-ARDS morbidity, and, in females, the absence of tissue factor on endothelial and some hematopoietic cells contributes to increased morbidity. We observed that the lung edema, measured by the lung index, was significantly higher in females lacking tissue factor. On going work, includes the analysis of gene expression for markers of coagulation, inflammation, and oxidative stress using qRT-PCR. Additionally, histopathological

analysis of lung tissues will be conducted. This study seeks to provide a comprehensive understanding of the pathology induced by malaria parasites in the lungs and aims to offer a basis for the development of therapeutic treatments against severe malaria.

46. MULTI-LOCUS SEQUENCE TYPING OF A DIVERSE COLLECTION OF PHYTOPHTHORA PALMIVORA ISOLATES AND THE NEWLY DESCRIBED P. HETEROSPORA – INSIGHTS INTO GLOBAL DISTRIBUTION

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Phytophthora palmivora is a plant-pathogenic oomycete with a cosmopolitan distribution and among the greatest host ranges in the genera, with over 160 host genera in 60 families, causing hundreds of millions of dollars in loss in hosts such as cacao and African oil palm. Phytophthora heterospora is the most closely related species to P. palmivora, having only been described in 2021 and has been found causing the same disease symptoms on crops that P. palmivora infects (olive, durian, Cattleya orchid, pomegranate). Currently, there have not been any studies on the distribution and host range of P. heterospora in relation to P. palmivora. In the present study, we assembled a diverse collection of isolates from 4 continents and over 50 hosts and conducted multi-locus sequence typing using three house-keeping genes (ITS, Btub and NADH). Our results indicate additional hosts for P.

heterospora and reveals insights into the distribution of the two species.

47. SPECIATION AND ECOLOGICAL PROFILING OF GIBELLULA FLORIDENSIS WITHIN DIVERSE ENTOMOPATHOGENIC COMMUNITIES

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Introduction: Entomopathogenic fungi play crucial roles in arachnid population dynamics, yet the speciation mechanisms and ecological interactions of these fungi remain underexplored. This study documents the identification of a new fungal species, Gibellula floridensis, from infected spiders, and examines its ecological niche and relationships within its community.

Method: Specimens were collected from spider cadavers in forested areas near the University of Florida, followed by fungal isolation and cultivation. We utilized morphological assessments and multi-locus molecular phylogenetic analyses to characterize the new species.

Results: Our research successfully identified Gibellula floridensis as a distinct species. Further ecological assessments through environmental DNA sampling from surrounding soil, leaf litter, and plant surfaces did not reveal additional Gibellula sequences, suggesting a highly specialized ecological niche. The study also mapped the distribution of various entomopathogenic fungi within these habitats, providing insights into their community dynamics.

Conclusions: The discovery of Gibellula floridensis contributes significantly to our understanding of fungal biodiversity and

speciation in entomopathogenic communities. This research highlights the species' specialized interactions and the importance of detailed ecological surveys to elucidate the environmental distributions and host-specificity of fungal pathogens.

48. THE INFLUENCE OF VEGETATION STRUCTURE ON THE PREVALENCE AND INTENSITY OF STRONGYLOID NEMATODES INFECTING NATIVE RODENTS IN AN AFRICAN SAVANNA.

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Introduction: The structure and complexity of vegetation cover influence a broad range of ecological processes. One of these processes is pathogen transmission, for which the influence of vegetation structure on host abundance and abiotic conditions can have important effects. Savannas, characterized by a heterogeneous vegetation structure of trees and grasses, are ideal systems to explore the mechanisms relating to disease and habitat structure. Furthermore, as land management regimes influence the structure of savannas, an understanding of the effects of vegetation structure on pathogen transmission becomes an issue of applied relevance.

Method: We assessed the cascading influence of vegetation structure, through host abundance and abiotic conditions, on the prevalence and intensity of Strongyloid nematodes infecting native rodents in a southern African savanna. We hypothesized that increased vegetation cover will increase nematode prevalence and intensity and relative host abundance due to protection from sun exposure and host predation. We tested our hypotheses at the Mlawula Nature Reserve in Eswatini with plots simulating different techniques of vegetation management. We used Sherman traps to capture rodents and fecal flotation to detect and count Strongyloid nematode eggs from collected fecal samples.

Results: Using data collected in 2023 and 2024, we found that canopy layer diversity had a significant relationship with nematode prevalence while shrub and grass cover had contrasting relationships with nematode intensity that varied depending on host species. We also found that relative host abundance had a significant relationship with grass cover; however, neither relative host abundance nor host sex influenced nematode prevalence and intensity.

Conclusions: The vegetation changes caused by different land management regimes affected parasite transmission and relative host abundance. Increasing canopy layer diversity may increase nematode prevalence by reducing sunlight exposure and improving survival; however, nematode intensity may vary between host species depending on how they interact with their environment. Further research is needed to investigate how different types of vegetation cover influence nematode intensity since understanding pathogen transmission in changing landscapes can help improve land management regimes in southern African savannas.

49. THE RELATIONSHIP BETWEEN PLACENTAL MALARIA INFECTION, HIV, INTESTINAL PERMEABILITY, AND INFLAMMATION IN POST-PARTUM KENYAN WOMEN

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Placental malaria, which compromises maternal health and causes poor infant health outcomes in association with Plasmodium falciparum infection, has long been associated with intense and damaging inflammatory responses in the maternal peripheral and placental blood. HIV infection further exacerbates these outcomes. Maternal responses to these infections lead to significant placental dysfunction, but how they impact other organ systems in pregnant women remains underexplored. The aim of this study was to determine the relationships between placental malaria (PM), HIV serostatus, markers for intestinal permeability (lipopolysaccharide (LPS), LPS-binding protein (LBP), and zonulin), and markers of inflammation (interleukin-6 (IL-6), IL-8, IL-10, Tumor Necrosis Factor (TNF), TNF receptor II (TNFRII), and interferon γ-induced protein 10 (IP-10) in post-partum women exposed to malaria and HIV in Kenya. Clinical and diagnostic parameters and levels of these biomarkers in the peripheral venous blood were analyzed. LPS and zonulin levels weakly to moderately positively correlated with placental parasite load, and zonulin was significantly elevated with PM and PM/HIV co-infection as well as self-reported recent malaria infection. LPS was elevated in women with low birthweight infants relative to normal birth weight infants, and positively correlated with markers of inflammation (TNF, IL-6, IL-8, IL-10,

and IP-10). These data suggest that infection-associated inflammatory cytokine and chemokine responses coincide with induction of gut permeability in pregnant women and may synergize to exacerbate poor maternal and infant outcomes. Future investigations to establish the causal relationships between these responses in malaria and HIV infected women could reveal potential new targets for diagnostics and therapeutic intervention.

50. THE THERMAL IMPACT ON PARASITE PERFORMANCE DATABASE

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Global climate change (GCC) and emerging infectious diseases are formidable ecological challenges that must be addressed as they have the combined potential to influence many aspects of global biology. Most GCC-caused declines and extinctions have been attributed to species interactions (e.g., host-parasite), highlighting the fact that science must develop an understanding of organismal responses to GCC to predict when GCC might trigger outbreaks of infectious disease. Current research has posited that GCC will increase many infectious diseases and reduce biodiversity, ecosystem services, and food production. To facilitate understanding the effects of GCC on host-parasite interactions, we

have developed a database called Thermal Impact on Parasite Performance (TIPP), consisting of the thermal performance of pathogens grown in culture or on hosts and vectors. We conducted a literature search in Web of Science and have extracted approximately 1600 thermal performance curves from 800 parasite species spanning helminths, fungi, bacteria, viruses, protozoa, and insects, as well as parasites of humans and wild and domesticated plants and animals; work is ongoing to collect additional data from Scopus and PubMed. Additionally, by combining TIPP with databases on the thermal performances curves of hosts (e.g., BioTraits and VecTraits) we expect to provide science with an accessible, central location for thermal performance data that can be used to test GCC-based hypotheses for temperature dependent interactions. In this poster, will outline the potential for TIPP to address key ecological and evolutionary questions such as: 1) how parasite thermal optimum relates to the host's preferred thermal or geographical range, and how thermal mismatches between host and pathogen might influence infection dynamics, 2) how phylogenetic relationships inform thermal performance curve shape, 3) whether the thermal performance curves of pathogens significantly differ among differing categories of host-parasite relationship (i.e., specialist vs. generalist parasites, invasive status, or low abundance species of conservation concern), and 4) how do thermal performance curves vary along major environmental gradients, such as altitude and latitude.

51. YOU ARE WHAT YOU EAT: BIDIRECTIONAL HORIZONTAL GENE TRANSFER REVEALS HIDDEN HISTORY OF HOST-PARASITE INTERACTIONS IN PLANTS

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Horizontal gene transfer (HGT) is a widespread genetic process shaping genome complexity. While being generally rare in eukaryotes, HGT is particularly abundant in the mitochondria of parasitic plants potentially due to the susceptibility of mitochondria to alien DNA and the intimate symbiotic relationship that facilitates gene exchange. Using a new bioinformatic tool, we discovered an unprecedented six thousand HGTs in the mitochondria genomes of the parasitic family Orobanchaceae. These HGTs make up to 25% percent of the mitochondrial genomes of the parasites and are strongly correlated with the host preference of the parasitic plant, suggesting parasitic plant diet can significantly influence their genomic composition. Phylogenetic analysis on HGT unfolded complex histories of species interaction including coincided host and range shifts and repeated transitions from generalists to specialists. We demonstrated that host-to-parasite HGT dominated but parasite-to-host HGT also occurred at appreciable frequency. Such bidirectional transfer creates multiple pathways for DNA to be shared in a multiparty symbiotic network, each leaving a unique phylogenetic signal. This explains a highly unusual case of HGT among the distantly related, sympatrically distributed parasites Cistanche and Cynomorium and their common amaranth host in the Eurasia desert.

52. MAJOR MEMBRANE PROTEIN OF MYCOBACTERIUM AVIUM SUBP. PARATUBERCULOSIS ACTIVATES IMMUNE AND AUTOPHAGIC PATHWAYS IN BOVINE MONOCYTE-DERIVED MACROPHAGES

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Mycobacterium avium subspecies paratuberculosis (Map), the etiological agent of Johne's disease in ruminants, challenges veterinary health and food safety. Despite partial immune control, Map persists in macrophages via poorly understood mechanisms. We investigated how the Map major membrane protein (MMP) modulates immune responses in bovine monocyte-derived macrophages. MMP, a key bacterial membrane component recognized in Johne's disease, is a critical antigenic target. High-resolution transcriptomics showed that MMP stimulation rapidly activates genes linked to pro-inflammatory cytokine signaling, antigen processing, and presentation via MHC I/II. Gene Ontology and KEGG analyses highlighted upregulation of TNF, IL-17, and NF-κB signaling, potentially fostering cytotoxic T cell development. Phosphorylation assays confirmed MAPK activation within minutes, implicating p38 and JNK1/2 in early responses. Machine

learning revealed subtle MMP-specific gene signatures such as ATG5 and ATG12, implicated in autophagosome assembly. These findings suggest a dynamic interplay between antibacterial autophagy and immunostimulatory pathways. Importantly, MMP may be a potential vaccine target, as it elicits immune-activating signals and engages host defenses restricting Map survival. Overall, this ex vivo framework delineates Map infection's molecular underpinnings, offering new insights into macrophage-based immunity and informing novel therapeutic and prophylactic strategies against paratuberculosis. Our data open avenues for translational studies, illuminating the interplay between MMP, macrophages, and protective host immunity.

53. MULTI-LEVEL PROTEOMICS REVEALS EPIGENETIC SIGNATURES IN BCG-MEDIATED MACROPHAGE ACTIVATION

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Introduction: The existing vaccine for tuberculosis (TB) is the BCG vaccine, which contains live attenuated M. bovis, the bovine strain of Mycobacterium tuberculosis. Exposure to M. bovis has shown to alter response to unrelated pathogens in a phenomenon known as trained immunity.

Trained immunity and epigenetic post-translational modifications (PTMs) are inextricably linked. This study combines multiple levels of proteomics data to elucidate a novel network of epigenetic effectors and markers in common across a global cell environment.

Methods: THP-1 monocytes were differentiated into macrophages, then exposed to M. bovis. The total and histone-focused proteomes were obtained using established methods (data at PXD051187), and the phosphoproteome data was retrieved from Choudhary et al. (PXD013171). Mass spectrometry data from a Q Exactive was processed with Proteome Discoverer and EpiProfile.

Results: Multiple peptides of interest in BCG application were identified as differentially expressed in infected cells. Additionally, components and products from numerous epigenetic reader complexes (NuA4, NuRD, NSL, Sin3A, SIRT2, SIRT6) were differentially present.

Conclusions: We identified potential mechanisms for BCG-induced trained immunity in macrophages, as well as target molecules for further investigation. Future work will involve making the bioinformatics pipeline publicly available, as well as identifying specific genetic targets of the proposed network.

54. PRETOMANID DOSING AND PHARMACOKINETICS IN NORTH AMERICAN PATIENTS WITH TUBERCULOSIS, 2019-2024

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Introduction: Pretomanid (PMD) is recommended for treatment of multi-drug-resistant tuberculosis (MDR-TB) in the regimen bedaquiline-pretomanid-linezolid with or without moxifloxacin (BPaL/M). However, data regarding PMD serum drug concentrations in clinical practice is limited.

Methods: PMD therapeutic drug monitoring (TDM) using liquid chromatography–tandem mass spectrometry was performed on plasma samples from North American patients submitted to the University of Florida Infectious Diseases Pharmacokinetics Laboratory. Adverse drug reaction and outcome data were not available.

Results: From November 2019 through October 2024, 151 patients from 78 locations contributed 385 PMD TDM samples. Median age was 39 years (range 11-92) and 56% were male. There were 177 unique TDM occasions, including 21 patients with more than one TDM occasion. Most patients (98%) received PMD 200mg daily or 5 days/week. PMD trough concentrations were measured on 145 samples (median 2.07 mg/L [range 0-9.18]), 2-hour post-dose concentrations on 84 samples (3.00 mg/L [0-7.93]), and 5-hour post-dose concentrations on 156 samples (3.74 mg/L [0.70-14.04]). Among 145 trough samples, 51 (35%) exceeded the expected target (1.0-2.4 mg/L) and 16 (11%) were below. Among 156 peak samples,

55 (35%) exceeded typical steady state range (2.3-4.3 mg/L) and 22 (14%) were below.

Conclusions: Using a 200mg daily PMD dose, most patients (86%) achieved or exceeded the target peak serum concentrations and a third of patients had measured trough levels above the expected range. Future studies should correlate peak PMD serum concentrations with MDR-TB treatment success and evaluate the impact of high trough concentrations on safety outcomes.

55. RECEPTOR-MEDIATED EFFECTS ALLOW FOR BETTER SELECTION OF B-LACTAM ANTIBIOTICS AGAINST MYCOBACTERIUM AVIUM COMPLEX

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Introduction: Mycobacterium avium Complex (MAC) is one of the most common nontuberculous mycobacteria (NTM) that causes severe pulmonary infections in immunocompromised patients. β-Lactams and β-lactamase inhibitors can be used combination therapy to treat bacterial infections including MAC.

Aim: This study aimed to identify target receptors and their occupancy patterns of 14 β-lactams and two β-lactamase inhibitors (BLIs) towards five penicillin-binding proteins (PBPs: PonA1, PonA2, FtsI [PBP2], PbpA and a DD-carboxypeptidase), three L,D-transpeptidases (LDTs) and one metallo-β-lactamase (MBL) using purified membranes of MAC strain ATCC 700898.

Methods: Bound receptors were fluorescently labelled and identified by proteomics. Confocal microscopy was used to record morphological changes. Drugs bound up to six receptors and clustered into five groups.

Results: Carbapenems and cefoxitin (cluster 1) inactivated most target receptors at low concentrations (≤0.125 mg/L). Ceftriaxone and cefotaxime (cluster 2) bound five receptors and did not bind one LDT. While, ceftaroline and penicillins (without mecillinam) (cluster 3) bound four receptors. Clavulanic acid, aztreonam ceftazidime (cluster 4) inhibited PonA1, whereas avibactam, mecillinam, carumonam (cluster 5) showed limited binding at clinically relevant concentrations. Penicillins and ceftazidime yielded filaments in MAC.

Conclusion: These first receptor-binding patterns enable rational choices of β-lactams as part of safe and effective combination therapies against MAC.

56. WHEN BUGS SPILL SECRETS: A GENOMIC AND MACHINE LEARNING ANALYSIS OF TUBERCULOSIS TRANSMISSION IN GHANA

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Introduction: Tuberculosis (TB) is a significant public health issue in Ghana, particularly in urban areas. Identifying the proportion of recent transmission due to high-risk behaviors is crucial for creating targeted interventions.

Methods: From June 2022 to June 2023, new and previously treated TB cases were recruited and enrolled at a tertiary hospital in Accra, Ghana. Recent transmission was defined by a bacterial genetic distance of 0 – 5 single nucleotide polymorphisms (SNPs). Bacterial genomes from culture-confirmed cases were analyzed using a supervised learning algorithm to estimate transmission probabilities. Individual reproductive numbers (Ri) were calculated, with transmitters defined as those with Ri values above one. A 1:2 matched case-control analysis measured the impact of HIV and substance use on recent TB transmission.

Results: Among 133 pulmonary TB cases; 72% were males, 17% had HIV, and 47% reported alcohol or drug use. High-quality SNP data from 80 culture-confirmed cases allowed the recreation of 9,926 case pairs, identifying 34 probable infectors. Ri values were similar between treatment naïve and previously treated cases, but previously treated men had higher Ri values than women (p = 0.024). Factors associated with being a probable infector included normal BMI (aOR = 4.8; 95% CI: 1.2 – 23.7) and HIV coinfection (aOR = 19.5; 95% CI: 1.9 – 257.5). Cases under 25 years old had higher odds of being probable infectors compared to those 45 and older. An interaction between HIV and hemoptysis (p = 0.051) suggested that HIV coinfection with delayed presentation increased transmission risk. Approximately 80% (PAF = 0.8; 95% CI: 0.3 – 1.0) of recent TB transmission events were attributable to HIV coinfection, with most HIV-positive cases diagnosed less than a year before TB diagnosis. Substance use was not linked to probable infector status.

Conclusions: This study is one of the first to use genomics and machine learning to analyze recent TB transmission in urban Ghana. By calculating individual reproductive numbers and assessing the impact of HIV on transmission, the study identified key clinical features, particularly HIV coinfection and delayed presentation, that drive recent TB transmission.

57. A DESCRIPTION OF TICK SPECIES AND THEIR RICKETTSIA SPP. COLLECTED FROM FLORIDA COYOTES (CANIS LANTRANS) IN RURAL AND URBAN ENVIRONMENTS

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Urbanization significantly impacts wildlife ecology, particularly in the interactions between wildlife species and ectoparasites. As habitats become fragmented and altered, urban-adapted species like coyotes (Canis latrans) increasingly thrive in urban environments, potentially serving as reservoirs for tick-borne pathogens (TBP). This study, in collaboration with USDA Wildlife Services, investigated the ectoparasite communities associated with coyotes in both urban and rural habitats in Florida, and focused specifically on the presence of Rickettsia species, including members of the Spotted Fever Group (SFG) Rickettsia. We identified tick species morphologically and identified Rickettsia species using molecular analysis. From 17 coyotes we collected 96 ticks. Of these 93 ticks were identified down to species, excluding one half-sample, which was missing the head and only identified to genus. 87 of these were extracted and tested for Rickettsia spp. 48/87 were positive for Rickettsia spp., with 17 of these testing positive for SFGR. Understanding the tick community present on coyotes is an important aspect of habitat ecology with regards to wildlife management. The potential risks posed by

coyotes introducing ticks into human dominated environments require further study as deforestation and human encroachment becomes more widespread.

58. ATP-SENSITIVE INWARD RECTIFIER POTASSIUM (KATP) CHANNELS REGULATE SALIVA PROTEIN SECRETION IN THE LONE STAR TICK (AMBLYOMMA AMERICANUM)

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Tick saliva secretion is essential for blood feeding and pathogen transmission. Understanding the molecular basis of tick salivary gland physiology is critical for developing novel intervention strategies to reduce disease transmission. Pharmacological activation of KATP channels by pinacidil was found to reduce fluid secretion from isolated salivary gland and blood ingestion in the lone star tick, Amblyomma americanum. Yet, the mechanism of reduced blood ingestion remained unknown. In this study, we conducted immunohistochemistry on the salivary gland throughout the tick feeding process. In Type II and III acini, which are responsible for fluid and protein secretion, KATP channel proteins were highly expressed in the off-host stage and early feeding phase (0-2 days) and were not expressed during later stages (6+ days) of feeding. In Type I acini, which are responsible for osmoregulation, KATP channel expression was absent in early feeding phases but increased in later feeding stages. Importantly, this temporal expression pattern throughout feeding was verified

with pharmacology where we observed reduced potency of KATP agonists to fluid secretion during later feeding stages. We also analyzed the protein components from tick saliva under the exposure of KATP channel activator pinacidil and blocker tolbutamide with LC-MS/MS and western blots. Of the 573 non-redundant proteins, 40 were differentially secreted after exposure to KATP activators or inhibitors. The proteomic data were validated with western blots where pro-tick feeding saliva proteins AV422, AAS27, and AAS41 were differentially secreted after exposure to KATP activators or inhibitors. Importantly, significant differences in blood ingestion were observed after exposure to modulators of KATP channels, suggesting that differential protein secretion after exposure to KATP modulators is correlated to changes in blood feeding behavior. Our data demonstrated that KATP channels have a dynamic expression pattern during the tick feeding period, which is likely attributable to the role of KATP channels in regulating pro-feeding saliva proteins required during tick early feeding phases.

59. CHANGING SEASONS OF DENGUE SUITABILITY IN FLORIDA: CMIP6 PROJECTIONS

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Introduction: Dengue fever, an infectious viral disease spread by Aedes spp mosquitoes, is not endemic to Florida, but this could change in the future. Changing temperatures could potentially expand the range of Aedes aegypti mosquitoes, putting Florida at increasing risk for dengue endemicity (year-round local transmission).

Method: We apply a thermal suitability model of dengue transmission by Aedes aegypti to predict monthly transmission suitability in Florida. We apply the model to baseline climate and then compare it to projected future scenarios based on a range of 5 CMIP6 general circulation models (GCMs). The 5 GCMs (HadGEM, ACCESS-CM2, CMCC-ESM2, MRI-ESM2-0, and MIROC6) predict temperature for 2021-2040 and 2041-2060.

Results: Results from analysis in R show that all 5 models predict an increase in months of thermal suitability for transmission for both future time periods compared to baseline climate. All models indicate that South Florida, including the highly populated Miami, will have increased months of suitability in the 2041-2060 scenario, with some areas subject to year-round suitability. The change in suitability between 2021-2040 to 2041-2060 is not consistent across the 5 models. These results indicate that while all of Florida is at increasing risk of dengue transmission suitability compared to baseline, different parts of the state will experience this differently.

Conclusions: Special consideration for vector control and surveillance measures should be afforded to South Florida and other populous regions most likely to experience significantly increased dengue suitability in the coming years.

60. CLIMATE INFLUENCES ON DENGUE SEASONALITY IN BRAZIL: A 16-YEAR STATE-LEVEL ANALYSIS

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Dengue transmission is significantly influenced by environmental factors such as temperature and precipitation, which affect mosquito breeding and virus transmission. In endemic regions, dengue outbreaks typically follow a seasonal pattern linked to environmental changes.

This study aims to quantify the seasonality of dengue in Brazil, characterize its year-to-year variation, explore shifts in its seasonal pattern over time, and assess correlations between dengue incidence and environmental variables (temperature and precipitation).

Daily time series data of reported dengue cases from Brazil's Notification Diseases Information System (SINAN) from 2000 to 2015 were analyzed. The seasonality was characterized by calculating the weighted mean day of the season for dengue incidence, temperature, and precipitation for each state and season. Linear models were fitted to assess trends in dengue seasonality over time, and correlation coefficients were estimated to capture year-on-year variation between dengue incidence and climate variables.

The analysis revealed substantial year-on-year variation in dengue seasonality across Brazilian states. Sao Paulo had the highest total

cases, peaking over 10,000 in multiple years, with notable seasonal peaks in March and April. The linear model showed no statistically significant trends in dengue incidence from 2000 to 2014. Temperature showed a slightly positive but inconsistent correlation with dengue incidence (mean correlation of 0.08), with only two states exhibiting significant associations. Precipitation had an even weaker average correlation (0.05), with only one state showing a significant positive relationship.

The seasonality of dengue incidence shows no clear temporal trend, suggesting the need for further analyses with alternative models or more data to capture potential seasonal or non-linear patterns. The weak and variable associations between climate factors and dengue incidence indicate that while climate may influence dengue seasonality, its effects are localized and inconsistent across regions.

61. COMBINING SINGLE CELL TRANSCRIPTOMICS AND PROTEOMICS TO IDENTIFY NOVEL ANTIFEEDANT TARGETS IN THE SOUTHERN GREEN STINK BUG

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The southern green stink bug, Nezara viridula, is a polyphagous pest of multiple agricultural crops, and the development of novel mechanisms to kill or to prevent feeding by this pest is needed. Stink bugs feed by inserting their piercing-sucking mouthparts into plant tissues through physically rupturing cells and secreting ‘gel’ saliva that forms a sheath around the stylets. In addition, ‘watery’ saliva containing digestive proteases and nucleases is secreted to digest plant cell contents prior to ingestion. Therefore, the stink bug salivary gland and secreted saliva are critical for feeding. Products that inhibit the function of secreted proteins or reduce the secretory activity of the stink bug salivary gland are likely to inhibit feeding. However, understanding of the cellular composition and druggable targets of stink bug salivary glands is incomplete. To address this gap, the aim of this study is to define the expression profile of individual cells of the accessory and principal salivary glands relevant to protein secretion. Whole salivary glands were dissected from a total of 10-15 stink bugs and pooled for each of three replicates. Whole tissues were then dissociated and single-cell sequencing of N. viridula salivary glands

performed. From the resulting data, 13 cell clusters were identified, with preliminary findings suggesting that 7 of these clusters are sub-sets of secretory cells each producing a unique profile of secreted digestive enzymes and effectors. Proteomics analysis of watery saliva and of gelling saliva was conducted to address whether the markers of secretory cell subsets could be identified in the secreted saliva. Initial analysis indicates that 1 of the 7 secretory cell clusters produces > 40% of matched salivary proteins in both watery and gelling saliva. Further, sub-sets of secretory cells each produce around 15-25% of either watery saliva or gelling saliva. Understanding the proportions of secreted salivary proteins originating from specific sub-sets of secretory cells permits the identification of putative targets to disrupt stink bug salivary gland secretory function and prevent insect feeding and consequent damage. This work provides the foundation for addressing whether there are sub-sets of secretory cells producing large amounts of specific proteins that could both be visualized using in situ hybridization and targeted using RNAi.

62. CONDITIONAL STERILITY IN TRANSGENIC AEDES AEGYPTI MALES: A NOVEL APPROACH FOR REDUCING MOSQUITO FERTILITY

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Aedes aegypti is the primary vector of dengue, zika, chikungunya, and yellow fever, posing major global health risks. Traditional control methods, such as insecticides, Sterile Insect Technique (SIT), and Release of Insects carrying a Dominant Lethal (RIDL) gene are becoming less effective. A new genetic control approach offers a promising alternative for population suppression. This study generated transgenic Aedes aegypti males using the I-Ppol nuclease gene to induce sterility by disrupting rDNA in the testes. Transgenic males were crossed with wild-type females for the experiment cross. The control reciprocal cross included transgenic females crossed with wild-type males. Fertility was assessed across four crosses. Female fertility was reduced by 70% in the first two crosses, with minimal impact on fecundity. The third cross showed a 78% fertility reduction, while the fourth showed a 48% reduction.

These results indicate that double-transgenic males significantly reduce female fertility, supporting their potential use in mosquito population control.

63. DELAYED MORTALITY AMONG CASES OF WEST NILE VIRUS ILLNESS IN FLORIDA, 2013-2022

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Introduction: Mortality associated with West Nile virus (WNV) disease is believed to occur mostly during acute or early convalescent phases of illness. Nationally, the reported mortality rates for WNV disease range from 3%-15%. Studies reviewing data from states besides Florida suggest mortality may occur for at least a year after acute WNV disease. This study aims to assess the occurrence of delayed mortality among Florida WNV disease cases and evaluate the associated mortality rate.

Methods: This analysis included 139 patients identified as WNV neuroinvasive disease cases in Florida residents reported to the Florida Department of Health (FDOH) through the Merlin reportable disease surveillance system from January 1, 2013, to December 31, 2022. Exact patient names and dates of birth from Merlin were utilized to search the FDOH Vital Statistics database for corresponding death certificates issued between January 1, 2013, and December 31, 2023. If a death certificate was not found, it was assumed that the individual was either still alive or passed away outside of Florida. Death certificates listing unspecified other mosquito-borne viral encephalitis, unspecified encephalitis, West Nile virus infection, sequelae of viral encephalitis, unspecified encephalitis, myelitis and encephalomyelitis, and unspecified encephalopathy as contributing causes of death were designated as directly related to the WNV infection. Delayed mortality was

defined as deaths that occurred ≥90 days after reported onset of illness.

Results: Death certificates were issued for 28 WNV cases, of which ten were directly attributed to WNV infection. The overall mortality rate for WNV was 7.2%. Among WNV-attributed deaths, the median interval from onset to death was 54.5 days (range: 15-723 days), with three deaths occurring beyond 90 days after onset. The median age at death was 76 years of age (range 55-85 years). Three of the ten deaths attributed to WNV were not recorded in Merlin. Of these, one occurred before the case closeout period, and two occurred afterward.

Conclusions: The results of this analysis suggest mortality due to WNV disease occurs beyond the acute or early convalescent phases of the illness and that the associated mortality rate in Florida is underestimated. More directly linking the vital statistics and Merlin databases would be instrumental in enhancing data quality and ensuring greater accuracy. Deaths that occur after the case closeout period are not documented in Merlin. Currently FDOH is investigating ways to capture WNV-related deaths outside of the reporting year to better document delayed mortality.

64. DETECTION OF HAEMOSPORIDIAN PARASITES IN WHITE IBIS AND TRICOLORED HERON NESTING AND ASSOCIATED MOVEMENT OF BIRDS

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Introduction: Avian malaria and related diseases, caused by haemosporidian parasites transmitted by blood-feeding vectors, are common infectious diseases in birds. However, infection dynamics in wading birds remain understudied. These species are capable of long-distance movement throughout their life cycles, increasing their exposure to a wide range of vectors.

Method: We collected baseline data on haemosporidian parasite prevalence from blood samples of white ibis (Eudocimus albus) and tricolored herons (Egretta tricolor) captured at breeding colonies in coastal Alabama, USA, from 2020 to 2022. A nested PCR method targeting the cytochrome-b gene was used to detect parasites from the genera Plasmodium, Haemoproteus, and Leucocytozoon. Satellite transmitters were deployed on individuals to track movements, and a Net Squared Displacement (NSD) method was used to classify individuals as residents or migrants.

Results: We found haemosporidian infection in 42.1% of white ibis ($n = 95$), significantly higher than the 14.5% infection rate in tricolored herons ($n = 69$) (Fisher's exact test, $p < 0.01$; odds ratio = 0.22; 95% CI: 0.07–0.61). Adult white ibises had a higher prevalence

(67.9%) than juveniles (31.1%) (Fisher's exact test, $p < 0.01$; odds ratio = 4.25; 95% CI: 1.87–10.49), consistent with findings from South Florida. Infected white ibis displayed migration routes along the northern Gulf Coast, while one infected tricolored heron wintered in Lake Nicaragua. Of the 50 cytochrome-b sequences longer than 400 bp, 26 (52%) were of sufficient quality for BLAST analysis, and all showed over 99% similarity to the Haemoproteus lineage EUDRUB01, previously reported in white ibis in South Florida and in scarlet ibises (Eudocimus ruber) at the São Paulo Zoo, Brazil.

Conclusions: Our findings suggest that breeding colonies act as transmission sites, with potential for additional transmission during the nonbreeding season. Estuarine habitats in the northern Gulf of America may serve as major transmission centers. There may be previously undocumented wintering sites shared by wading bird populations from both Alabama and Florida. Future research should prioritize testing vector competence and mapping detailed distributions of vector alongside bird movement to better understand infection dynamics.

65. DEVELOPMENT AND ASSESSMENT OF AEDES AEGYPTI SURVEILLANCE METHODS

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Background: The Aedes aegypti mosquito is commonly known as the Yellow Fever Mosquito. Along with Yellow Fever (YFV), the Aedes aegypti mosquito is known for being the natural vector of several deadly arboviruses including dengue (DENV), chikungunya (CHIKV), Rift Valley (RVF) and Zika (ZIKV). The WHO reports that as of 2020, DENV alone is responsible for an estimated 40,000 deaths and 96 million infections yearly. Due to the habitat and range of the Aedes aegypti mosquito, more than 3.9 billion people across 129 countries are at risk of Ae. aegypti transmitted arboviruses. However, this number is expected to increase. Ae. aegypti territory is estimated to grow by 19.96 million km2 by 2050 in accordance with forecasted increasing temperature patterns. As global temperatures rise, more people are expected to be at risk for serious arboviral infections, making their invasive proliferation a serious threat to global public health. Adequate surveillance of Ae. aegypti populations and behavior will be crucial for the development of appropriate public health intervention.

Objective: To harmonize and standardize existing immature Aedes aegypti collection methods in order to develop a finalized surveillance protocol. This protocol will be tested for reproducibility using a pilot launch and later rolled out as part of an expandable surveillance system.

Methods: Standardizing immature collection methods was done by performing a series of benchmark tests, comparing the efficiency of novel net designs and collection protocols against traditional methods. Following the creation of a robust collection and surveillance protocol, a pilot surveillance trial was launched in Borbón, Ecuador. The study population included 200 randomly selected households from a participant population, which were visited and inspected for the presence of immature Aedes aegypti mosquitoes in water receptacles.

Results: Benchmark testing revealed a 668% increase in novel immature mosquito collection methods compared to traditional collection methods. The pilot surveillance trial revealed significant vulnerabilities in the reproducibility of our protocols, including data capture issues, specimen labeling inconsistencies, and irregularities in protocol execution across groups.

Conclusion: Following the pilot period, protocols were rectified and data collection improved with surveillance rollout. The finalized surveillance system will yield reliable monthly data on mosquito ovipositing behavior and vertical DENV transmission.

66. ENHANCING ARBOVIRUS SURVEILLANCE AND RISK MANAGEMENT IN THE PUBLIC HEALTH SYSTEMS OF GEORGIA, TURKEY, AND UKRAINE

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Mosquitoes Aedes aegypti and Aedes albopictus are the main transmitters of viruses that are becoming widespread and affecting humans. These mosquitoes are two main vectors of Chikungunya -, Dengue -, and Zika- viruses, whereas various Culex mosquito species transmit West Nile virus. They are expanding their home ranges and spreading the above-mentioned viruses into countries they weren't found in previously. In recent years, both - Ae. aegypti and Ae. albopictus, invaded countries around the Black Sea Region. Their distribution is largely unknown.

Controlling mosquito numbers and virus surveillance methods are the principal methods to reduce disease transmission. We aim to improve arbovirus surveillance by characterizing the relative mosquito species composition in select countries of the Black Sea region - Georgia, Turkey, and Ukraine. A virological component of our project involves collection of entomological and clinical samples to test for Chikungunya-, Dengue-, West Nile-, and Zika-

viruses. Collectively, this project will develop an integrated mosquito management program, improve virus surveillance, and decrease the risk of virus transmission.

To-date, mosquitoes were collected during 2023, 2024 in 5 regions of Georgia:

West Georgia: Adjara, Guria and Samegrelo regions; East Georgia: Kakheti region; and South Georgia: Samtskhe-Javakheti region.

Morphological identification revealed that, Ae. albopictus and Culex spp. had been collected. So far, none of the four target viruses have been detected by RT-qPCR in the mosquitoes that were collected. However, clinical studies to identify the incidence and seroprevalence of selected arboviruses in patients from different hospitals revealed five imported cases of Dengue Fever, which were diagnosed by RT-PCR.

Turkey: Mosquito samples were collected from 338 locations across 15 cities during two seasons (May-September of 2023 and 2024).

Morphological identification revealed: Ae. albopictus, Cu. pipiens, and other species were collected in years 2023 and 2024.

RT-qPCR screening of mosquito pools from years 2023 and 2024 yielded negative results for Chikungunya-, Dengue-, West Nile-, and Zika- viruses, suggesting that these viruses were not circulating in the tested mosquito populations at the time of collection.

Ukraine: mosquito samples were collected from a total of 83 locations around the city Odesa, Odesa and Mykolaiv regions from August to October of 2024.

Morphological identification revealed: Ae. albopictus, Cu. pipiens, and other mosquito species had been collected.

67. EPIDEMIOLOGICAL AND CLINICAL OUTCOMES OF HUMAN MONOCYTIC EHRLICHIOSIS (HME) PRESENTING TO A SINGLE CENTER UNIVERSITY HOSPITAL IN THE STATE OF FLORIDA, USA.

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Background: Human monocytic ehrlichiosis (HME) is a vector-borne disease caused by Ehrlichia species. Transmission in the United States through the bite of a lone star tick (Amblyomma americanum). Serious complications can arise which includes the development of hemophagocytic lymphohistiocytosis (HLH).

Methods: We conducted a retrospective analysis of those with HME from 2004 to 2023 who presented to our tertiary, university-based hospital centre in Gainesville, Florida, USA. Inclusion criteria consisted of positive Ehrlichia polymerase chain reaction (PCR) assay, or Ehrlichia serology reactivity or pathologic evidence of a morulae on blood smear. Epidemiological, clinical and outcome data were collected. HLH 2004 diagnostic guidelines were followed in which 5 out of 8 criteria most be fulfilled.

Results: Forty-nine cases of HME were identified with 65.3% (N=32) being Male. Median age between 51 to 76 years old with 14% (N=7) under fourteen. Most (N=35; 71.4%) patients presented to the hospital from April to September. Common symptoms (Figure 1) include fever (N=37; 75.5%), headache (N=21; 42.8%) and myalgia (N=17; 34.6%). Tick exposure reported by most (N=35; 71.4%). Physical exam findings reported include abdominal tenderness (N=13; 26.5%), maculopapular rash (N=12; 24.4%) and acute

encephalopathy (N=6; 12.2%). Seven patients (14.2%) were immunocompromised (solid organ or bone marrow transplantation or immunosuppressive medications). Forty-four (89.8%) required hospitalization with a median overall length of stay of 7 days. Thirteen (26.5%) required medical intensive care unit (MICU) care. Median days to start doxycycline were 2 after arrival with 13 patients starting more than 5 days after initial clinical assessment (Figure 2). Diagnostic testing included forty-seven having reactive Ehrlichia serologies, eleven with Ehrlichia positive PCR, and five where morulae was identified on blood smear. The majority of infections were due to E. chaffeensis (N=47; 95.9%) but two were identified as E. ewingii/canis. Eight patients developed HLH (Table 1.) with an incidence rate of 16.3%. Forty-eight fully recovered but one patient with orthotopic liver transplantation died from complications.

Conclusion: This is the largest single centre investigation in Florida evaluating HME. We found that HLH should be considered in those with this infection. HME is a significant vector-borne disease that needs prompt recognition among health care providers.

68. GENERATIONAL PERSPECTIVES ON MALARIA PREVENTION AND THE ROLE OF HEALTH MESSAGING: INSIGHTS FROM BIOKO ISLAND, EQUATORIAL GUINEA

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This study examines trends in malaria health communication and its impact on malaria prevention behaviors in Malabo, Equatorial Guinea, using data from the Bioko Island Malaria Elimination Project (BIMEP). The findings show that television remains the dominant source of malaria health communication, though there is a noticeable shift toward more localized communication methods such as home visits and hospital consultations, particularly in peri-urban and rural areas. Generational differences in media consumption are evident, with older individuals preferring traditional media and younger individuals increasingly using interactive platforms for malaria information. Logistic regression models reveal that older household heads are more likely to own and perceive access to long-lasting insecticide-treated nets (LLINs), though health communication significantly improves LLIN ownership and access across all age groups. These results emphasize the need for adaptive, multi-channel health communication strategies that consider regional, generational, and media-specific preferences to enhance malaria prevention efforts.

69. IN VITRO RESPONSES OF PATHOGENIC AND NON PATHOGENIC RICKETTSIA TO INCREASED OSMOLARITY

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Rickettsia are Gram negative obligate intracellular bacterial pathogens vectored to mammals by arthropods. Rickettsia parkeri and R. montanensis are Spotted Fever Group (SFG) species and pathogenic and non pathogenic, respectively. R. montanensis is usually found in the American Dog tick (Dermacentor variabilis) which is also a vector for R. rickettsii, the causative agent of Rocky Mountain Spotted Fever. R. parkeri is vectored by the Gulf Coast tick (Amblyomma maculatum). During the zoonotic cycle, Rickettsia are subjected to changing osmolarity and salt concentrations as the bacteria reside in human blood and immune and endothelial cells compared to tick hemolymph, ovaries, midgut and saliva. In the tick, the midgut fluctuates between ~300 and ~600 mOsmoles during blood meals, then returns to ~300 mOsm when feeding concludes.

When infected into Vero cells, we observed enlarged plaques for both R. parkeri and R. montanensis when the overlay osmolarity was increased with either sodium chloride or sodium sulfate. However, when we used glycerol to increase overlay osmolarity, we only observed enlarged plaques for R. montanensis, R. parkeri plaques remained the same size. This suggests that while R. montanensis responded to the increase in osmolarity, R. parkeri may respond to sodium specifically. We performed targeted qPCR for several genes of interest- known virulence factors including proteins involved in motility and invasion (rickA and sca2), the

type 4 secretion system (virB4) and metal transporters gltP and MC1_RS03805). At 3 days post infection sustained in medium with increased osmolarity, we observed the greatest change in gene expression for sodium sulfate compared with unmodified tissue culture medium for R. parkeri. Expression data was normalized to rpoD expression. We observed increased gene expression for motility genes for sodium chloride and sodium sulfate conditions and increased T4SS gene expression and metal transporter for sodium sulfate. We are currently using RNA-seq to examine the entire transcriptome for R. parkeri infected Vero cells in the increased osmolarity infections.

One gene we are investigating is a potential potassium transporter which shows homology to the Kup family transporter in Bacillus subtilis (KimA). Bioinformatics reveal that the MC1_RS03805 protein exhibits 24.2% identity and 42.7% similarity with KimA. The predicted tertiary structure aligns with the KimA crystal structure and key residues required for function are conserved. We cloned MC1_RS03805 into pBAD24 and transformed into E. coli deficient in potassium uptake. Preliminary data suggests that MC1_RS03805 may improve growth of these mutants in low potassium minimal medium.

70. INTEGRATING ANIMAL MOVEMENT SIMULATIONS AND DISEASE VECTOR DISTRIBUTION MODELING TO INFORM HEMORRHAGIC DISEASE MANAGEMENT IN FARMED DEER POPULATIONS

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Hemorrhagic viruses transmitted by Culicoides biting midges pose a significant threat to deer populations and are a critical challenge in wildlife management. Traditionally, integrated pest management approaches that incorporate information on hosts and vectors often struggle with limited data and are driven by reactive strategies, often failing to anticipate and mitigate disease transmission effectively. This research develops an innovative decision support tool that combines animal movement simulation informed by real data with species distribution modeling of disease vectors to provide a framework of simulated scenario exploration and proactive management insights.

This study focuses on farmed white-tailed deer, Odocoileus virginianus, a key species in many agricultural and wildlife systems, frequently impacted by vector-borne diseases. We developed a comprehensive simulation framework using GPS collar data to generate realistic animal movement patterns and space utilization distributions. Additionally, spatial modeling of

Culicoides biting midge occupancy was used to generate disease vector distributions across the landscape.

Our methodological approach integrates multiple advanced techniques and involves extracting movement patterns from empirical GPS collar data using step selection analysis, mapping species distribution for biting midges from count data at sampling locations and creating an interactive Shiny application for scenario exploration and risk assessment. By combining host and vector information, this research helps to identify areas of high potential for disease based on animal utilization distributions and vector distribution. Preliminary analysis demonstrates the tool's potential to provide spatially explicit, dynamic risk assessments that can guide targeted management interventions.

Our integrated simulation approach coupled with an interactive decision support tool offers a novel, data-driven method for understanding and mitigating hemorrhagic disease risks in deer populations. The developed decision support platform represents a significant advancement in interdisciplinary disease management, bridging ecological modeling, epidemiology, and wildlife management. By synthesizing complex data streams from animal movement patterns, disease vector distributions, and landscape characteristics, the tool provides a friendly interface to understand disease risk, allowing users to develop proactive interventions that can potentially minimize disease transmission.

71. LEVERAGING THE NICHE CENTRALITY HYPOTHESIS TO MODEL THE GEOGRAPHICAL PATTERNS OF ABUNDANCE OF THE SOFT TICK ORNITHODOROS TURICATA AMERICANUS, A VECTOR OF EPIDEMIOLOGICAL CONCERN IN NORTH AMERICA.

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The soft tick Ornithodoros turicata is a vector of tick-borne relapsing fever and has been identified as a potential vector of African Swine Fever Virus, the pathogen responsible for an ongoing global epizootic that threatens agroindustry worldwide. Recent surveys show that O. turicana americanus (OTA), the eastern population of this tick, occurs across a large portion of Florida, with its range overlapping areas of high abundance of feral pigs. These surveys also suggest the existence of ample variation in the abundance of OTA. Given the pivotal role played by vector abundance in disease dynamics, we set to model the abundance of OTA across its distribution in the eastern USA. We used a statewide OTA survey to assess two hypotheses regarding the drivers of its abundance: i) abundance is influenced by the local environmental characteristics such as vegetation cover or topography, and ii) the

similarity of the environmental conditions of a site to those of the species niche center is positively correlated to abundance. Bayesian models of abundance that accounted for sampling biases clearly supported the second hypothesis. Our work suggests the utility of the niche center hypothesis to model vectors and gain insights into diseases systems. Furthermore, this work advances our understanding of the ecology of OTA and highlights areas where feral swine and OTA co-occur at high abundances. These areas might be of special management importance in an eventual introduction of African Swine Fever Virus to the USA.

72. MAPPING DENGUE DYNAMICS IN PUERTO RICO: CLUSTER PATTERNS ACROSS TIME, SPACE, AND A DECLINING POPULATION

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Dengue remains a persistent public health threat in Puerto Rico, characterized by endemic transmission, frequent outbreaks, and a 3-to-7-year epidemic cycle. Recent years have seen increasingly severe outbreaks, culminating in the declaration of a public health emergency in early 2024. Ongoing human population decline has introduced a new demographic context for interpreting spatial disease risk, with meaningful implications for where clusters form and persist across the island. This study examines the spatiotemporal epidemiology of dengue in Puerto Rico from 2010 to 2023 by exploratory spatial data analysis (ESDA) methods to assess cluster detection. The retrospective space-time scan statistic was applied in SaTScan to identify statistically significant high-rate dengue clusters across all 78 municipalities. Annual dengue case

data were obtained from the Puerto Rico Department of Health at the municipality level. Municipality boundary shapefiles were sourced from the Government of Puerto Rico GIS Portal. Centroid coordinates for each municipio were derived from the U.S. Gazetteer internal point data. Annual population estimates were obtained from the U.S. Census Bureau's American Community Survey. The Poisson model was implemented at a 25% PAR. To complement this analysis, Differential Local Moran's I, a local indicator of spatial autocorrelation (LISA) comparing each year to the previous, was applied across the study period. This provides further opportunity to examine the spatial and temporal consistency of hotspots, cold spots, and spatial outliers across the study period. The Poisson model identified a large primary cluster in the western coastal region and two smaller secondary clusters in the central region, all of which were early in the study period. Results from the LISA and population density analyses include the identification of temporally consistent clusters and spatial outliers, offering insight into how dengue's spatial patterns have shifted alongside population loss. Together, these approaches offer a more comprehensive understanding of dengue's changing geography in Puerto Rico while underscoring the strengths and limitations of different ESDA methods. This work highlights the need for flexible, data-informed tools in vector-borne disease surveillance.

73. MEASURING HUMAN IGG RESPONSES TO AEDES SALIVARY RECOMBINANT PROTEIN IN A BRAZIL COHORT

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Vector-borne diseases are a large cause of human mortality and morbidity worldwide. Aedes aegypti is a known vector of several viruses including yellow fever virus, dengue virus, chikungunya virus, and Zika virus. Traditional methods of measuring mosquito exposure including trapping protocols and human responses to salivary gland homogenate are often challenging to implement and standardize. Our objective was to measure human IgG responses to Aedes mosquito salivary proteins (MSP) and correlate the association between antibody responses to MSP with individual characteristics like CHKV serostatus and spatial location.

We conducted a longitudinal analysis of serum samples from 241 participants in Pau de Lima, Salvador, Brazil. The collection of samples was done in two timepoints: L44 (Sept-Nov 2019) and L46 (Jul-Oct 2021). To quantify antibodies to mosquito salivary protein IgG responses, we used an enzyme-linked immunosorbent assay (ELISA) with Aed a D7 as coating antigen. D7 protein is a major salivary protein secreted by female mosquitos.

On average, we found an increase on optical density measurements (i.e., an increased immune response) between the two time points, indicating a higher exposure risk to Aedes mosquitoes. The results demonstrated that OD responses were negatively correlated with age in both time points. We also found that responses were spatially correlated in both rounds of measurement. However, we did not find that antibody responses to the D7 were associated with serological status of CHIKV infection.

74. METAGENOMICS TARGETING BACTERIAL SPECIES IN I. PERSULCATUS AND H. ASIATICUM IN MONGOLIA COLLECTED IN 2020 AND 2022.

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Introduction: Tick-borne pathogens have been detected in various tick species throughout Mongolia, posing significant risks to Mongolian herders, who make up about 26% of the population, and face an increased risk for exposure to ticks. Bacteria such as Anaplasma spp., Borrelia spp., Coxiella spp., Ehrlichia spp., Francisella spp. and Rickettsia spp. have been detected in ticks throughout the country. While Dermacentor nuttalli has been

extensively studied, gaps remain in our understanding of the other two most common tick species in Mongolia, Ixodes persulcatus and Hyalomma asiaticum.

Methods: To characterize bacterial species in I. persulcatus from Selenge and H. asiaticum from Dornogobi, ticks were screened using Oxford Nanopore Next-Generation Sequencing (NGS). Ticks were collected in 2020 via flagging and dragging and 2022 via hand collection, respectively, and were morphologically identified. Following homogenization and DNA extraction, tick DNA was subjected to 16S bacterial amplification via PCR. NGS was performed on the PromethION using the Native Barcoding Kit. Positive NGS results were confirmed using conventional PCR and Sanger sequencing.

Results: Sample testing is currently ongoing.

Conclusion: This study aims to address gaps in bacterial surveillance by characterizing the bacteria in I. persulcatus and H. asiaticum in Mongolia. Continued surveillance and pathogen testing will enhance our understanding of public health risks posed by bacterial pathogens and contribute to the knowledge of pathogen distribution in these two provinces.

75. MODE OF TOXICITY OF THE B-TRIKETONE LEPTOSPERMONE TO AEDES AEGYPTI MOSQUITOES

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Leptospermone, a naturally occurring β-triketone and the main component of manuka oil (Leptospermum scoparium), is a known plant HPPD inhibitor. It was found to produce rapid knockdown and substantial toxicity in adult Aedes aegypti when applied topically or through tarsal contact, with LD50 values of 150 ng per mg of mosquito and 357 ng per cm2, respectively. Despite its strong toxicity to mosquitoes, leptospermone was non-toxic to ticks, honeybees, and fruit flies, indicating a high degree of insect specificity. Moreover, it was equally lethal to non-blood-fed and blood-fed mosquitoes, suggesting that its mechanism of action is unrelated to HPPD inhibition. Molecular modeling showed close structural similarities between leptospermone and mammalian sulfonamide carbonic anhydrase (CA) inhibitors. Follow-up in vitro

potency assays using mosquito midgut homogenates or purified CA confirmed that leptospermone inhibits Ae. aegypti CA but not mammalian CAs. Carbonic anhydrases are metalloenzymes involved in tissue pH regulation, and although they are distributed throughout insect tissues, they are especially abundant in the mosquito midgut. This prompted an investigation into whether leptospermone affects midgut pH regulation. Indeed, leptospermone significantly lowered midgut pH in Ae. aegypti compared to control mosquitoes, supporting the hypothesis that leptospermone's insecticidal effect involves CA inhibition. These findings establish leptospermone as an effective mosquitocide, causing rapid knockdown and mortality in Ae. aegypti at doses approaching those of natural pyrethrins, even in pyrethroid-resistant strains. Furthermore, the results indicate that leptospermone's mode of action targets CA—a novel mosquitocide mechanism distinct from its mode of action in plants.

76. MODELING FUTURE DENGUE TRANSMISSION SUITABILITY IN NEPAL USING CMIP6 CLIMATE SCENARIOS

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Dengue, first reported in Nepal in 2004, manifested large outbreaks in 2022 and 2023 (54,784 and 51,243 cases), with cases reported in all 77 districts. Initially confined to the lower plains, dengue has spread to higher elevations and is now endemic. Aedes aegypti and Aedes albopictus mosquitoes are competent dengue virus vectors in Nepal. Using previously published temperature-driven transmission suitability models, we mapped baseline and projected future areas and duration of transmission suitability for dengue transmission for both mosquito vectors.

CMIP6, the latest Coupled Model Intercomparison Projects (CMIP), contains future climate scenarios generated by different modeling groups. Shared Socioeconomic Pathways (SSP) are emission scenarios based on various socioeconomic assumptions. We obtained baseline and CMIP6 projected temperature for 2021-2040 (commonly referred to as 2030) from WorldClim.org for SSP245 and SSP585 using the five CMIP6 models: HadGEM3, ACCESS CM2, CMCC ESM2, MRI ESM2 0, and MIROC6. These were analyzed under two Shared Socioeconomic Pathways, SSP245 and SSP585, for the periods representing 2030 and 2050. Under baseline conditions, 54 percent and 59 percent of Nepal's land area, approximately 148,000 square kilometers, was suitable for dengue transmission by Aedes aegypti and Aedes albopictus, respectively.

By 2030, projected suitability increases to 64 to 65 percent for Aedes aegypti and 60 to 61 percent for Aedes albopictus, with further increases expected by 2050. These trends emphasize the impact of climate change on vector habitats and transmission risks.

In a changing climate, historically colder areas of Nepal are becoming more temperate, expanding suitable areas for Aedes albopictus transmission upward in elevation, while warmer regions are becoming suitable for longer periods for the more heat-tolerant Aedes aegypti. Our mapped monthly transmission suitability under baseline and projected temperatures illustrates the contrasting seasonal shifts of the two vectors. This study is the first step in unpacking the role of changing temperatures in shaping dengue transmission seasons in a country of extreme topographic and climatic variation, already facing two vectors with differing temperature-dependent transmission profiles.

77. MODELING HOST-PARASITE INTERACTIONS IN MALARIA BLOOD-STAGE INFECTIONS IN RHESUS MACAQUES

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Introduction: Malaria is globally the most deadly parasitic disease in humans, and the long-time coexistence with malaria has left indelible marks in the human genome that are the causes of a variety of genetic disorders. Anemia is arguably the most common and severe complication of malaria, yet the root causes and mechanisms involved in the pathogenesis of malarial anemia are unclear and very difficult to study in humans. Non-human primate (NHP) model systems enable the mechanistic study and quantification of underlying, causative factors of malarial anemia, and particularly the onset of severe anemia.

Materials and Methods: A discrete recursive model was developed to simulate host-parasite interactions during the blood stage infection; it accounts for reticulocytes, red blood cells (RBCs), and infected RBCs. The parameters of this mechanistic model were optimized against the readouts of individual macaque data, which had been obtained in the course of Plasmodium coatneyi and P. cynomolgi infections of cohorts of malaria-naïve rhesus macaques

(Macaca mulatta). The model allowed detailed estimations of the levels of erythropoietic output, reticulocyte lifespan, RBC removal, and the immune response against the parasite in each macaque.

Results and Discussion: The results showed that rhesus macaques have a response to a P. cynomolgi infection that is difficult to understand: As expected, the infection resulted in anemia, yet 60% of the RBCs were lost by a mechanism other than parasite invasion, which is known as bystander effect. To compensate for the severe anemia, the host released younger reticulocytes and increased the erythropoietic output. These responses, however, appeared to be poorly coordinated, as the release of younger reticulocytes occurred too early, namely, while anemia had not yet set in, thereby probably aiding the parasite more than the host. Additionally, increased production of RBCs was only detected after treatment that lowered the parasitemia. The model also showed that, similarly to humans, reticulocytes in rhesus macaques circulate for about 24h before becoming mature RBCs.

Conclusions: Anemia, as a sequela of malaria, was due in 60% to bystander destruction of RBCs, and by an inability of the host to up-regulate erythropoiesis before suppression of parasitemia.

78. MODELING PEPTIDE INSERTION SITES ON SALMONELLA TYPHIMURIUM FOR SURFACE DISPLAYING

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Outer membrane proteins (OMPs) play critical roles in bacterial structure, function, and host interaction. OmpA in Escherichia coli is a heat-modifiable protein that anchors the outer membrane to the bacterial cell wall, contributing to membrane stability under varying environmental conditions. It has strong structural integrity, as peptide insertions into cell-surface-exposed regions of OmpA do not disrupt export or membrane assembly. The ompA gene in Salmonella typhimurium exhibits similar characteristics, indicating similar export and import mechanisms between the two organisms. However, the extracellular domains of OmpA in S. typhimurium differ significantly from those in E. coli, potentially offering unique sites for peptide display that may serve as novel vector regions for antigen presentation. This study aims to explore this potential of the four main loops of S. typhimurium OmpA as an efficient and stable platform for peptide insertion, with implications for vaccine development. Models of S. typhimurium OmpA were produced through PyMOL. PLZ4 was inserted into different locations on the main four extracellular loops. Loop two insertion in position “A,” between lysine and glycine, was revealed to have the most potential for a vaccine vector position. This position best presents the PLZ4 insertion to the outside without interfering with other loops or the membrane. Additionally, more of the PLZ4 is exposed on the top of the loop for position A than other positions in loop two. This study provides a platform for

further research into OmpA loop two for vaccine development with potentially improved safety and efficacy.

79. MOSQUITO BIODIVERSITY IN A PATHOGEN ENDEMIC SETTING IN HAITI

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Introduction: Mosquito-borne diseases remain a significant public health challenge in Haiti, with mosquito species such as Aedes aegypti and Culex quinquefasciatus acting as key vectors for arboviruses and lymphatic filariasis. Understanding mosquito biodiversity in urban settings can provide insights into species co-occurrence and environmental drivers of diversity, hence its relevance to the field of community ecology in addition to implications of biodiversity and community composition for vector control. This study examines mosquito biodiversity and species interactions in a highly urbanized area of Haiti.

Methods: Mosquitoes were collected between August 2018 and September 2019 using Biogents Sentinel, CDC Gravid, and CDC Light Traps at 19 study locations within Haiti's Ouest Department. Species were identified morphologically, and biodiversity was assessed using alpha diversity, Shannon's, and Simpson's indices. Boosted regression trees (BRTs) were used to evaluate environmental drivers of diversity, incorporating static and temporally varying covariates such as temperature, precipitation, vegetation indices, and human population density. Species co-occurrence networks were generated to explore interspecies relationships, using both a crossproduct network and a probabilistic network.

Results: A total of 22,504 mosquitoes including six total species were captured, with Culex quinquefasciatus (53.4%) and Aedes aegypti (16.9%) being the most abundant species. Biodiversity metrics varied significantly by trap type, but not by month. BRTs identified human population density, temperature, and vegetation indices as key predictors of mosquito diversity. Co-occurrence networks revealed significant positive associations between Aedes species and Cx. quinquefasciatus, while Psorophora columbiae exhibited negative associations with multiple species.

Conclusions: This study highlights the ecological complexity of mosquito biodiversity in Haiti, emphasizing the role of human population density and environmental factors in shaping mosquito communities. The findings underscore the need for diversified trapping methods and further research on interspecies co-occurrence dynamics to inform vector control efforts in pathogen-endemic settings.

80. OCCURRENCE AND GENETIC CHARACTERIZATION OF TICK-BORNE RELAPSING FEVER BORRELIA SPP. IN A FLORIDA SUBSPECIES OF SOFT TICKS (ORNITHODOROS TURICATA AMERICANUS)

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The threat of vector-borne diseases has continually increased in the past century and has necessitated additional research into the ecology of these vectors and their pathogens. In the southwestern United States, the occurrence and potential for disease spread of Tick-Borne Relapsing Fever (TBRF) has been studied extensively. In Florida, the only recorded occurrence of the Borrelia pathogens that cause TBRF was in two domestic dogs (Canis lupus familiaris) in the 1990s. Despite this discovery, further research was not pursued to identify the distribution or prevalence of this pathogen in the state. Additionally, Florida boasts a substantial population of the soft tick, Ornithodoros turicata americanus, that is capable of transmitting Borrelia. The goal of our study was to fill the

knowledge gaps regarding this disease system in the state. Our objectives were to 1) describe the presence and distribution of Borrelia spp. in Florida ticks; 2) phylogenetically describe the pathogen species compared to the western B. turicatae and other TBRF-Borrelia pathogens. We pooled ticks by sample location and extracted DNA from over 3,000 ticks systematically collected throughout the state. Conventional PCR was used with a genus-wide IGS primer to detect any Borrelia spp. present in the ticks. We Sanger sequenced 7 pools (7/580; 1.21%) that were positive for Borrelia spp. Three samples were a 99.7%, 98.25%, and 96.9% match for B. turicatae, two samples showed >99% identity for both B. turicatae and B. venezuelensis, and the final two showed percent identity of 97% and 95.2% for B. venezuelensis, respectively. Thus, sequencing results did not provide a definitive conclusion for which Borrelia spp. was present in Florida but suggest that TBRF Borrelia in soft ticks is diverse and potentially represents multiple species. To fully understand the diversity of Borrelia spp. in the state, whole genome sequencing and a Multilocus Sequence Typing (MLST) strategy will be used to identify the potentially diverse Borrelia pathogens that were discovered in these soft ticks. This strategy will allow us to identify housekeeping and commonly sequenced genes throughout the bacterial genome that will help to differentiate these species and provide a better understanding of the phylogenetic relationship between them. By uncovering the pathogens and their prevalence in the state, we can better understand and mitigate the risk of this vector-borne disease for humans and companion animals in Florida.

81. OROPOUCHE IN FLORIDA

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Background: Oropouche fever (OROV) is an emerging vector-borne disease that has spread through the Caribbean and the Americas, including Cuba. Cuba has recently been experiencing an Oropouche outbreak, which puts Florida at an increased risk. Florida has the largest Cuban population in the United States and regularly sees imported cases of vector-borne diseases, from Cuba. OROV is spread through the bite of an infected biting midge (Culicoides paraensis) or mosquito (Culex quinquefasciatus). Both vector types are found within Florida, which increases the potential for local transmission .

Methods: OROV cases were reported in Florida Department of Health's state reportable disease data system, Merlin. OROV testing was performed by both the Florida Department of Health (FDOH) and Centers for Disease Control and Prevention. Both confirmed (PCR positive) and probable (antibody positive) cases were included in this analysis. While OROV is not specifically listed as a reportable disease in Florida, arboviral diseases not otherwise specified are listed as reportable. ArcGIS Pro 3.1 was used to create a frequency map showing OROV cases by county. Microsoft Excel was used to create other data visualizations and to perform statistical analysis.

Results: In Florida, 103 cases of OROV have been reported as of February 1, 2025. The first case was reported in July 2024. Most cases had an onset in July with 52 reported cases. Miami-Dade County (n=61 cases) and Hillsborough County (n=16) saw the

greatest amount of OROV cases. All cases reported travel to Cuba during their exposure period and almost all cases (98%, 101) were traveling to visit friends/relatives. The most affected age group was 35-64-year-olds (63%, n=65), followed by 65-year-olds and older (17%, n=18), 18-34-year-olds (15%, n=15), and 0-17-year-olds (5%, n=5). The median age of OROV cases was 52.5-years-old. Males accounted for 55% of the cases. White Hispanics were the most affected, making up 86% of all cases. The most reported symptoms were fever (94%), headache (80%), myalgia (78%), chills/rigors (67%), fatigue (67%), diarrhea (55%), arthralgia (54%), nausea/vomiting (52%), and malaise (39%).

Conclusion: When Cuba experiences a vector-borne disease outbreak, Florida experiences an increased risk due to Florida's large Cuban population, suitable climate, and vector types. Frequency of OROV cases were higher among counties with a larger Cuban population such as Miami-Dade, and all cases had direct ties to Cuba, either traveling to visit relatives or having recently moved to Florida from Cuba. Due to this increased risk, continued monitoring of OROV activity in Cuba will be important. Communities with a high population of travelers frequenting Cuba would also be good targets for increased vector bite prevention education, ongoing enhanced case finding activities, and vector control surveillance activities.

82. PERCEPTIONS, ATTITUDES, AND PREFERENCES OF AMERICAN CATTLE PRODUCERS REGARDING MESSAGING ABOUT TICK-BITES AND TICK-BORNE DISEASES

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Introduction: Haemaphysalis longicornis, an invasive tick first reported in the U.S. in 2017, threatens livestock by transmitting Theileria orientalis and causing severe health issues such as anemia and mortality. Its spread is driven by rapid reproduction and broad host range. Effective control requires both mechanical and chemical methods, along with increased awareness and education among cattle producers to ensure adoption of preventive practices and reduce risks to animal health and agricultural productivity. This study aimed to assess the effectiveness of different social media communication strategies in raising awareness among U.S. cattle producers about tick-borne disease risks and preventive actions.

Methods: The study surveyed 116 cattle producers in the Southeastern U.S. using a quasi-experiment with mock social media posts, employing a 2×3 factorial design to evaluate the impact of message sources (Extension agent vs. cattle producer) and message framing (prevention-oriented, fear-based, neutral) on engagement, perceptions (fear and attitudes toward risk management), and preventive behaviors.

Results: Results show that cattle producers were most likely to engage with social media posts through quick actions like reacting with emojis while commenting less frequently. Posts from fellow cattle producers, especially those framed as prevention-oriented or fear-based, saw higher engagement, while neutral messages received the least interaction. Additionally, prevention-oriented messages led to higher concern about tick risks and more proactive attitudes toward managing them, while fear-based messages resulted in lower post perceptions, and neutral messages were effective in prompting preventive actions, particularly when shared by an Extension agent. Social engagement is positively associated with cattle producers' perceptions of tick risks and their willingness to take preventive actions. Moreover, attitudes toward tick risk management showed a stronger correlation with preventive behaviors than fear, showing the role of cognitive evaluations over emotional responses.

Conclusions: These findings highlight the importance of carefully framing messages to encourage engagement and foster preventive behaviors. Overall, actionable, prevention-oriented content, particularly when delivered by trusted sources, appears more effective than fear-based appeals in promoting health-related behaviors among agricultural audiences.

83. PREVALENCE OF DIROFILARIA IMMITIS AND PLASMODIUM RELICTUM IN CULEX QUINQUEFASCIATUS POPULATIONS ACROSS FLORIDA

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Mosquito-borne diseases, particularly those transmitted by Culex quinquefasciatus, represent a persistent global health threat, affecting both human and animal populations. Among the key pathogens carried by this mosquito are Dirofilaria immitis (dog heartworm) and Plasmodium relictum (avian malaria). These diseases significantly impact wildlife, with avian malaria having resulted in the extinction of several endemic bird species, and dog heartworms costing billions annually in control methods. In Florida, where Culex quinquefasciatus is widespread, the prevalence of these pathogens raises concerns for conservation efforts, making it critical to monitor the spread of these diseases in mosquito populations. This study employed quantitative PCR (qPCR) with a COX1 primer-probe set and an 18S primer set to detect P. relictum, as well as D. immitis-specific primers to detect and quantify dog heartworm. Additionally, next-generation sequencing (NGS) was used to enhance pathogen detection and provide a comprehensive understanding of their distribution. Culex quinquefasciatus mosquitoes from six Florida counties were screened. The qPCR results yielded 12 P. relictum positives and 2 D. immitis positives, while NGS identified several additional P.

relictum cases not detected by qPCR. This demonstrates the superior sensitivity of NGS in detecting P. relictum. The findings confirm the utility of qPCR for quantifying dog heartworm and highlight the potential of NGS as a powerful tool for surveillance, aiding in the early detection and control of these vector-borne diseases.

84. RABIES EXPOSURE CASE INVESTIGATIONS IN POLK COUNTY, FLORIDA 2016-2023: POTENTIAL FACTORS ASSOCIATED WITH A GREATER THAN TWO-FOLD INCREASE IN ANIMAL BITE REPORTING DURING 2021-2023

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Introduction: Rabies is an invariably fatal viral disease among mammals, including humans. The Florida Department of Health in Polk County (FDOH-Polk) investigates reports of animal bites among residents or their domestic animals that are received from healthcare providers, county animal control, and through self-reporting. During 2016-2020, FDOH-Polk investigated an annual average of 739 animal bites. However, beginning in 2021, the average number of animal bites reported increased more than two-fold. Descriptive epidemiology was performed to identify possible factors contributing to this increase.

Method: Data from the FDOH-Polk animal bite database maintained using Microsoft Access (version 2408) or Excel (version 2408) were reviewed to determine the number of reported and investigated cases by animal type and animal vaccination status. Merlin, the FDOH web-based reportable disease surveillance system was reviewed to identify cases that met the “Rabies Possible Exposure” definition, and hence that post-exposure prophylaxis (PEP) was indicated. Positive laboratory results from animals

submitted for rabies testing by FDOH were reviewed by type and year using Merlin. Lastly, the ESSENCE-FL syndromic surveillance system was utilized to gather emergency room data on bite-related visits among Polk County residents. All data were analyzed using R 4.4.0.

Results: From 2016 to 2020, FDOH investigated an average of 739 animal bite reports annually. Starting in 2021, this number more than doubled, with an average of 1600 reports investigated. The increase in bite reports involving domestic animals was particularly pronounced between 2021 and 2023. Data from ESSENCE-FL showed a stable trend in bite-related visits from 2016-2023, with an average of 1636 visits annually. A significant portion of these visits was classified as 'other', which included insect bites and cases with vague descriptions such as animal bites and rabies vaccinations. When classified by animal type, domestic animals were responsible for most bite-reported visits. In the Merlin system, raccoons, bats and foxes were identified as the primary animals involved in all laboratory-confirmed cases. Despite the increase in animal bites reported to FDOH from 2021-2023, there was no corresponding rise in cases where PEP was recommended.

Conclusion: The dramatic increase observed in animal bite reporting beginning in 2021 could be attributed to a change in practice by animal control in determining which bites to report to FDOH-Polk. Methods to improve the current bite reporting and investigation processes and reduce the volume of reported cases likely not meeting definition of "a potentially rabid animal" are being explored.

85. RESPONSE TO THE FIRST IDENTIFIED LOCAL TRANSMISSION OF DENGUE FEVER IN ORANGE COUNTY, FL — 2024

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Background: A recent substantial increase in dengue cases has been reported globally, particularly in the Americas. Dengue virus is transmitted to humans through the bite of infected mosquitoes, primarily Aedes aegypti and Aedes albopictus. Dengue fever is frequently asymptomatic or a mild illness but can present with severe illness. In 2023, 16 cases of travel-associated dengue were reported in Orange County, Florida. To date, in 2024, 48 cases have been identified, a 200% increase. On August 9, 2024, the Florida Department of Health in Orange County (FDOH-Orange) received a laboratory report of a commercial IgM positive result for dengue virus. The case-patient first experienced symptoms consistent with dengue on August 4. Subsequent investigation identified an additional symptomatic household member who experienced symptoms the same day. Neither reported any recent travel in-state nor internationally. An epidemiologic investigation and community risk mitigation response were conducted in coordination with Orange County Mosquito Control (OCMC).

Methods: The objectives of the investigation were to identify a potential index case, secondary cases, and to conduct enhanced active surveillance in the surrounding neighborhood. Passive syndromic surveillance for suspect arboviral illnesses in emergency department and urgent care facilities chief complaint

(CC) and discharge diagnoses (DC) is routinely conducted daily. Per guidance from the FDOH-Orange, OCMC conducted response activities, which included daytime and nighttime adulticidal, larvicidal spraying, vector prevention assessments, and a door-to-door mosquito prevention educational campaign.

Results: Testing by RT-PCR confirmed both initial case-patients to have DENV-3. case-patients reported no recent travel history. The case-patients anecdotally report contact with a symptomatic, recent traveler to Cuba—an adjacent neighbor. A total of 11 nearby households were surveyed and prevention education provided. From August 7 to October 7, 29 arboviral illness CC/DC were investigated, and no further dengue cases have been confirmed, either travel-associated or locally acquired.

Conclusions: This is the first confirmed case of locally acquired dengue in Orange County, Florida with subsequent evidence of a second household case identified. While previously present in Florida, the virus was eliminated from the United States several decades ago. Since then, a small number of imported cases from dengue-endemic countries have been reported. Imported dengue fever cases have drastically increased in the past year in Orange County, increasing the risk for local transmission. Rapid epidemiologic investigations and proactive environmental response by vector control partners are important to contain possible further local transmission when identified.

86. RHIPICEPHALUS SPP. TICK HABITAT SUITABILITY IN EAST AFRICA: INSIGHTS INTO ENVIRONMENTAL DRIVERS

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Introduction: Ticks are arthropod vectors of high public health and veterinary significance, transmitting a range of diseases that pose considerable challenges in Sub-Saharan Africa. The spread of tick-borne diseases (TBDs) results in significant livestock deaths and economic losses annually. The distribution of ticks is influenced by environmental factors that affect their survival and spread. Understanding these environmental drivers is crucial for informing effective disease and vector management strategies. Although East Africa bears a high prevalence of TBDs, there is limited research on the relationship between ticks, TBDs, and environmental conditions. This study aimed to examine the environmental factors that influence Rhipicephalus spp. tick distribution across five East African countries.

Methods: Occurrence data for ticks was sourced from a systematic review of georeferenced records on ticks and TBDs in East Africa, focusing on data from 1981 to 2019. Environmental covariates,

including climate, soil properties, and land cover type, were used at a 1km resolution. Pseudoabsence data was generated by selecting locations outside a 1km buffer around presence points. A convex hull was used to delineate the study area extent. Multicollinearity was assessed, and variables with low variance inflation factors (VIF) were retained. After cleaning and assessing data quality, 177 occurrence points were used in the analysis. A boosted regression tree (BRT) model was applied, with 5-fold cross-validation and an 80/20 split for training and testing.

Results: The model achieved an AUC of 0.75, indicating moderate predictive accuracy and a reasonable ability to discriminate between suitable and unsuitable habitats. Land cover emerged as the most important variable (48.84%), with settlement and agriculture areas showing the highest predicted probability of tick presence. Other key variables included soil organic carbon (13.75%) and annual mean temperature (9.05%). A suitability map was generated to highlight regions with the highest predicted probability of suitable tick habitats.

Conclusion: This study emphasizes the significant role of environmental factors in determining tick habitat suitability. The findings provide valuable insights for further research and targeted interventions in tick-borne disease management. These results are particularly relevant to public health agencies and disease control programs in East Africa, offering a framework for assessing tick habitat suitability considering ongoing environmental changes.

87. STRESSED OUT: A MURINE MODEL FOR INVESTIGATING MITOTEMPO'S IMPACT ON OXIDATIVE STRESS IN PLACENTAL MALARIA

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Introduction: Malaria is a bloodborne disease caused by Plasmodium parasites, transmitted through the bite of female Anopheles mosquitoes. Pregnant women infected with Plasmodium falciparum are more likely to miscarry and have higher rates of premature delivery, low birth weights and neonatal death. In 2020, 120.4 million pregnancies were at risk of complications from P. falciparum infection, and 85.2 million pregnancies were at risk of complications from infection with P. vivax (NIH). This disease is highly inflammatory, causing increases in oxidative stress as the immune system releases reactive oxygen species (ROS) to damage parasites. Since mitochondria are a major source of ROS production, targeting mitochondrial oxidative stress may help mitigate malaria-related complications. MitoTEMPO is a drug that directly scavenges ROS, specifically in the mitochondria.

Method: To evaluate its potential as a therapeutic to treat malaria during late pregnancy, we established a murine model by infecting pregnant BALB/c mice with Plasmodium berghei ANKA GFP (10^6) on Embryonic day (E) 13.5. From E 15.5-17.5, MitoTempo was administered once-daily via oral gavage. The experiment was terminated on E 18.5, and placentas were collected for RNA extraction. We then used qPCR to quantify relative levels of gene

expression of oxidative stress gene markers, including SOD-1, SOD-2, SOD-3 and CAT (superoxide dismutases 1, 2, 3 and catalase).

Results and Conclusions: We expect MitoTEMPO to decrease expression of oxidative stress gene markers due to its function as an antioxidant in previous studies with oxidative stress in cancer. This experiment could reveal a new drug to help treat placental malaria, as MitoTEMPO's potential as a malaria therapeutic is unclear.

88. A GLOBAL ANALYSIS OF BACTERIAL GENOMES LINKS SECONDARY METABOLISM AND ANTIBIOTIC RESISTANCE.

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A rapid rise in resistant pathogenic infections coupled with the decline in the development of new antibiotics makes the immediate discovery of novel antimicrobials of critical importance. Bacteria are prolific producers of secondary metabolites, many of which contributed to the 'Golden Age' of antibiotic discovery in the mid-20th century. There has been a resurgence in interest in identifying and isolating novel secondary metabolites synthesized by bacterial biosynthetic gene clusters (BGCs), given their known therapeutic potential. Although BGCs producing antimicrobial compounds may also house corresponding resistance genes, a quantitative link between BGCs and resistance remains unclear. To address this problem, we have developed a comparative genomic pipeline to mine bacterial genomes for BGC families and resistance genes in 10 well-studied taxonomic groups (including Mycobacterianales, Pseudomonales, Kitastatosporales, etc.). Genomes for all groups were retrieved

from the NCBI Genbank database and Natural Products Discovery Center and mined for BGCs using antiSMASH. BiG-SLiCE was used to cluster BGCs. CARD's (Comprehensive Antibiotic Resistance Database) Resistance Gene Identifier software and Resfams were used to identify genome-specific resistomes. By leveraging these bioinformatics tools, we discovered a complex association between secondary metabolism and bacterial resistance that is evident at the species level and influenced by bacterial ecological and evolutionary dynamics. Through statistical methods and analyzing the co-occurrence of resistance within BGCs, we have identified effective drug discovery strategies for various drug classes. Through phylogenetic reconstructions and tree-based metrics, we profiled the likelihood of horizontal gene transfer of resistance genes and BGCs across genomes. This analysis has (1) highlighted the drug discovery potential beyond Streptomyces, (2) assisted in identifying the optimal discovery pipeline for various drug classes, and (3) shed light on the evolutionary mechanisms that have shaped this complex relationship across different bacterial taxa.

89. A MOLECULAR BREEDING SOLUTION TO ENHANCE SOYBEAN DISEASE RESISTANCE

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Introduction: The average annual economic loss due to soybean diseases in the U.S. was about $4.5 billion from 1996 to 2016. We are using a molecular breeding approach to induce an enhanced immune response in soybeans (Glycine max). We have transformed soybeans with the Arabidopsis L-type Lectin Receptor Kinase-VI.2 (AtLecRK-VI.2), an extracellular NAD(P) receptor. Two independent homozygous lines have already been identified. We are planning to test relative expression of the transgene in these lines and test resistance to bacterial blight caused by Pseudomonas syringae pathovar glycinea, a globally relevant soybean disease.

Methods: Agrobacterium-mediated transformation was used to generate transgenic plants with the plasmid pFGC5941 that contains P35S:AtLecRK-VI.2. Genomic DNA of soybean plants was extracted with a CTAB method, and Polymerase Chain Reaction (PCR) was then used to confirm transgenic and single insertion lines in the T0 and T1 generations, respectively. Lines are currently being segregated to determine homozygosity, and quantitative PCR will be used to assess relative gene expression. Finally, disease resistance will be tested with P. syringae pv. glycinea containing the luxCDABE operon to facilitate luminescent bacterial titer measurements.

Results: Out of six transgenic plants generated in the T1 generation, four independent single insertion lines were identified, and from them, two independent homozygous lines were isolated. Preliminary data shows that both the average seed mass and number of seeds produced per plant for both homozygous lines are lower than nontransgenic control plants. Additionally, T2/T3 homozygous plants from line 3-7 are significantly smaller compared to the control plants.

Conclusions: We've shown that a CTAB genomic DNA extraction protocol coupled with a standard Taq-based PCR can confidently be used to identify segregants of transformed soybeans. While overexpression of pattern recognition receptors, such as AtLecRK-VI.2, has been shown to enhance disease resistance, they may also have a negative effect on plant growth. Previous research has shown that overexpressing AtLecRK-VI.2 in Arabidopsis induces a stunted autoimmunity phenotype, suggesting that high levels of this receptor can negatively affect growth and development. However, overexpression in a different species, Nicotiana benthamiana, or tobacco, showed no significant development changes. From our preliminary results, it seems likely that homozygous soybean lines overexpressing AtLecRK-VI.2 may have some stunted growth and/or reduced seed yields compared with wild-type plants; however, we still need to determine relative expression levels before final conclusions are made.

90. ALTERED CATHEPSIN EXPRESSION IN PATIENTS WITH BACTERIAL VAGINOSIS

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Introduction: Bacterial vaginosis (BV) is a common vaginal infection caused by a shift in microbial composition from Lactobacillus crispatus dominated to an anaerobic polymicrobial composition. Studies have shown significant associations between BV and an increased risk of acquisition and transmission of sexually transmitted infections and diseases including chlamydia, gonorrhea, HPV, and HIV. Shifts in the vaginal microbiome not only impact health but may also affect tissue remodeling processes by altering the expression of proteases including cathepsins. Cathepsins are proteolytic enzymes which play an important role in tissue remodeling by degrading extracellular matrix. Dysregulated cathepsin activity can have a pathogenic effect leading to abnormal degradation of the extracellular matrix, invasion of immune cells, and inflammation. Several disease states including HIV infection have been linked to an upregulation of cathepsins. Relevant to the vaginal microbiome, cathepsins K, L, S, and V are produced by immune and epithelial cells. Cathepsins K and L primarily degrade collagen types I and IV, while cathepsins S and V primarily degrade elastin. Here we investigated whether BV is associated with altered expression of cathepsins K, L, S, and V. We hypothesize that BV-related pathogenic bacteria increase cathepsin secretion, comprising the vaginal tissue and promoting associated disease states.

Methods: Vaginal fluid samples were collected from 6 BV positive and 6 BV negative patients with diagnosis was determined by a nurse practitioner using Amsel's criteria. Metagenomic next-generation sequencing was performed to identify abundances of microbes within vaginal fluid samples. Community state types (CSTs) classify vaginal microbiome composition. Microbiomes dominated by Lactobacillus crispatus were defined as CST I, Lactobacillus gasseri wre CST II, Lactobacillus iners were CST III, diverse bacteria were CST IV, and Lactobacillus jensenii were CST V. Western blotting and zymography were used to analyze expression and activity of cathepsins K, L, S, and V. Densitometry of bands were analyzed using ImageQuant TL software. Cathepsin expression and activity was evaluated for BV diagnosis and for most abundant bacteria present.

Results: In this study, results showed that expression of procathepsin K and procathepsin L were increased (7-fold, $p < 0.05$ and 2-fold, $p = 0.0798$, respectively) in BV positive vaginal fluid, and procathepsin V expression was decreased (2-fold, $p < 0.05$) in BV positive vaginal fluid. Despite altered expression of inactive cathepsin precursors, mature cathepsin expression did not vary between BV positive and negative diagnoses. 3 patients were classified as CST I, 2 patients were classified as CST III, and 7 patients were classified as CST IV. Mature cathepsin expression was shown to vary between these three community state types.

Conclusion: Shifts in vaginal microbiome composition and BV influence expression of proteolytic enzymes called cathepsins. Understanding how the vaginal microbiome and bacterial vaginosis influences tissue remodeling processes is important to develop novel preventions and therapeutics to reduce transmission of STIs including HPV and HIV.

91. ANTAGONISM OF ANTIBIOTIC ACTIVITY BY TOLFENPYRAD REVEALS INSIGHT INTO ITS MOLECULAR MECHANISM OF ACTION

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Francisella tularensis is a highly virulent Gram-negative bacterial pathogen that can be transmitted via aerosols and arthropod bites. Known as one of the most infectious pathogens, as few as 10 viable bacteria can cause a disease with upwards of 60% mortality in humans and animals. Thus, it is essential to identify new antimicrobials and improve existing treatment strategies against Francisella. To this end, we recently identified a new antimicrobial, tolfenpyrad, that potently blocks Francisella growth; however, its mechanism of action is currently unknown. Furthermore, it is unclear if tolfenpyrad modulates the activities of clinically-employed antibiotics. Knowledge of these relationships may elucidate tolfenpyrad's mechanism of action and improve current treatment strategies. By quantifying how tolfenpyrad affects the activity of well-characterized antimicrobials, we identified a series of antagonistic and synergistic drugs. Polymyxin B exhibits strong synergism with tolfenpyrad, likely by damaging the bacterial cell wall to facilitate uptake. In contrast, tolfenpyrad attenuates the activity of aminoglycosides and tetracycline. These antibiotics require a membrane potential for uptake, suggesting that tolfenpyrad may interfere with the electrochemical gradient

by inhibiting the electron transport chain. By directly measuring tetracycline internalization into F. novicida, we demonstrated that tolfenpyrad antagonizes tetracycline activity by reducing uptake. These data suggest that tolfenpyrad may block the formation of an electrochemical gradient across the bacterial inner membrane. These results inform our understanding of the molecular mechanism by which tolfenpyrad reduces Francisella viability and identifies tolfenpyrad-antibiotic combinations that are be beneficial or detrimental to therapeutic use of tolfenpyrad.

92. CAS6 INFLUENCES THE VIRULENCE OF PORPHYROMONAS GINGIVALIS

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Introduction: Periodontal diseases (PD) result from an abnormal immune response to dysbiotic microbial communities, progressively destroying tooth-supporting tissues. Porphyromonas gingivalis (Pg), a Gram-negative anaerobe, is strongly linked to PD due to its ability to dysregulate immunity and shape the subgingival microbiome under chronic inflammation. Recent evidence suggests a role for CRISPR-Cas systems in PD, but mechanisms remain unclear. While CRISPR-Cas typically defends against bacteriophages and mobile genetic elements, emerging data indicate its involvement in oxidative stress resistance, virulence, and immune system evasion. Our previous work showed that cas3 deletion alters the host response to Pg, yet the impact of other CRISPR-Cas genes remains unknown.

Method: Here, we examined the role of cas6 (Class 1 Type I CRISPR-Cas) in Pg virulence and macrophage inflammatory responses. Using classical antibiotic protection assays and colony counts, we assessed Pg intracellular survival in macrophages and transcriptomic analysis of THP-1 cells post-intracellular invasion evaluated gene expression. To determine the consequences of changes in the expression of genes involved Macrophage extracellular traps (METS) formation, assays were performed to quantify elastase from the co-cultures.

Results: Results revealed that Δcas6 Pg exhibited increased intracellular survival in macrophages compared to WT Pg. Transcriptomics identified 389 differentially expressed genes in THP-1 cells co-cultured with Δcas6 Pg, including genes associated with MET pathways. Notably, Δcas6 Pg induced significantly higher MET formation than WT ($p<0.05$, one-way ANOVA). These findings highlight cas6 as a factor in modulating the host inflammatory response and enhancing MET formation, underscoring its role in Pg virulence and host-pathogen interactions.

93. CHARACTERIZATING THE RESISTOME OF ESCHERICHIA COLI ISOLATED FROM FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA, USA

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Introduction: Antimicrobial resistance (AMR) is a critical public health threat, driven by the extensive use of antimicrobials in agriculture, human, and veterinary medicine. In Florida, white-tailed deer (WTD) farming plays a vital role in the economy and environment, but the use of antimicrobials in farmed WTD, along with their proximity to urban and agricultural areas, in-creases the pressure for AMR development. Understanding the resistance patterns in these deer populations is crucial for their health, as well as for wildlife and ecosystems. This research aimed to investigate the resistome of Florida-farmed WTD. Escherichia coli,

a commonly used indicator bacteria, was chosen to study AMR due to its pathogenicity and ease of cultivation.

Methods: Samples were collected from various tissues during necropsy, and a high-throughput NovaSeq sequencing system was used to analyze the E. coli genome. The AMR++ v 3.0 pipeline and ResistoXplorer tool were employed for data normalization and analysis.

Results: A total of 362 unique ARGs were identified, conferring resistance to 12 antimicrobial classes via 19 mechanisms. The most abundant classes were ß-lactams, multidrug resistance, and bacitracin. Antimicrobial susceptibility testing showed that 30% of E. coli isolates were resistant to at least one drug under aerobic conditions, while 68% were resistant under anaerobic conditions. Moreover, 15% of isolates displayed multi-drug resistance in both conditions. The study also compared genotypic and phenotypic AMR profiles using kappa, revealing good to very good agreement for several drugs.

Conclusions: Continued surveillance of key indicator ARGs can help predict overall AMR abundance, making resistance tracking crucial, particularly for drugs with intermediate resistance phenotypes. Selective antimicrobial use is recommended for those with high genotypic-phenotypic concordance and overall ARG prevalence.

94. DISCOVERING A NEW CLASS OF ANTIMICROBIALS THAT INHIBITS RESPIRATION FRANCISELLA

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Tularemia is a deadly disease caused by Francisella tularensis, an emerging intracellular bacterial pathogen that can be disseminated rapidly through aerosols and vector-borne transmission. Recent surveillance data demonstrate an increasing incidence in several countries. Although clinical isolates of Francisella species are sensitive to antibiotics, engineered or horizontal acquisition of antibiotic resistance is a constant threat to public health. Therefore, the identification of novel antibiotics that target previously undrugged pathways is required to safeguard human health. We recently identified that a widely used pesticide, tolfenpyrad, potently blocks Francisella growth; however, the antibacterial mechanism of action is undefined. In this study, we investigated the Francisella-targeting activity of tolfenpyrad and found that it disrupts ATP synthesis by inhibiting the electron transport chain (ETC). In F. novicida, the ETC is the primary energy-producing pathway, in whichthe oxidation the NADH leads to ATP production. Our data suggest that tolfenpyrad blocks ATP synthesis by inhibiting NADH oxidation.. This activity if limited to

species within Francisella and is highly potent. Unfortunately, tolfenpyrad is slightly toxic, limiting its candidacy as a therapeutic antimicrobial. To address this, we conducted a structure-activity relationship study and discovered tolfenpyrad derivatives with reduced toxicity and significantly enhanced antibacterial activity. These compounds block the intramacrophage growth of F. novicida and pathogenesis in an in vivo arthropod model of infection. Taken together, these findings suggest that tolfenpyrad is a promising therapeutic antimicrobial candidate that has a novel mechanism of bacterial inhibition in Francisella.

95. GENETIC EVOLUTION OF V. PARAHAEMOLYTICUS AND V. VULNIFICUS FROM AQUATIC BIRDS BROWN-HEADED GULL (CHROICOCEPHALUS BRUNNICEPHALUS) AND SEAWATER RESERVOIRS IN THAILAND

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Background: Vibrio vulnificus and Vibrio parahaemolyticus are naturally occurring inhabitants of estuarine and marine environments worldwide, responsible for a high number of deaths due to undercooked seafood consumption all over the world. Aquatic birds and seawater in Thailand are reservoirs for these bacteria, and it has been shown that migratory birds widely carry V. parahaemolyticus by consuming marine products. Therefore, they are a cause of the spread of these bacteria worldwide. The objective of this study is to apply a phylogeography framework to reconstruct the dispersal history, spatial and temporal dynamics of V. parahaemolyticus and V. vulnificus from an estuarine area in the province of Samut Prakan, Thailand.

Methods: A total of 94 novel isolates of V. parahaemolyticus were sequenced using MiSeq Illumina, from seawater (n=50) and aquatic birds' stools (n=44), Brown-headed gull (Chroicocephalus brunnicephalus), during nine months (August 2016 - April 2017) in the Bang Pu recreational center, Samut Prakan, Thailand. The new isolates were added to an initial global collection of 500 publicly-available V. parahaemolyticus complete genomes from environmental and clinical sources. Samples were accompanied with sufficient metadata and quality filtered for downstream analysis. We constructed a core genome alignment and SNP calling was performed using the reference genome V. parahaemolyticus RIMD 2210633. Maximum-likelihood (ML) phylogenetic tree from no-recombining SNPs using IQ-TREE v2.2.3 and Bayesian phylogenetics analysis confirming temporal signal was confirmed in TempEst v1.5.348 and reconstructed using a Bayesian phylogeographic approach in BEAST v2.

Results: Our analysis of a global collection of 500 clinical and environmental reveals the dynamics and expansion of V. parahaemolyticus. Our 94 novel isolates of V. parahaemolyticus from bird and seawater sources allowed us to confirm the widespread presence of Vibrios in aquatic reservoirs in the province of Samut Prakan, Thailand. This study revealed compelling evidence supporting the role of migratory birds and seawater as carriers of V. parahaemolyticus and V. vulnificus through marine products. The temporal scope from August 2016 to April 2017, allowed a comprehensive understanding of the evolutionary dynamics of these bacterial species. The ML and phylogenetics analysis show high genetic diversity of V. parahaemolyticus, which are broadly intermixed and divided into separate lineages.

Conclusion: V. parahaemolyticus and V. vulnificus are present in aquatic reservoir in Thailand. Therefore, continuous surveillance in the region is essential, and further study of environmental isolates is necessary to gather fundamental information about the source of enteric bacterial infections in the region. In addition, we will focus on investigating antibiotic-resistant genes in V. parahaemolyticus and V. vulnificus from birds and water sources, addressing the urgent need to understand the evolutionary mechanisms of resistance.

96. GENOMIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF BURKHOLDERIA PSEUDOMALLEI ISOLATES FROM VIETNAM BASED ON WHOLE-GENOME SNP BASED PHYLOGENETIC ANALYSIS

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Introduction: Melioidosis is a neglected tropical disease caused by the Gram-negative environmental saprophytic bacterium, Burkholderia pseudomallei. The disease is widely distributed globally but it is typically associated with tropical climates. Most human and animal infections are the result of exposure to contaminated soil and water environments. The disease and pathogen distribution has been highly studied throughout hyperendemic regions in Thailand and Northern Australia. However, many southeast Asian countries have a noteworthy, but underreported, burden of disease. In Vietnam, melioidosis is often underreported due to non-specific clinical presentations, limited diagnostic capacity, and gaps in public health infrastructure.

Method: This study investigates the phylogenetic diversity of B. pseudomallei isolates across Southeast Asia and Oceania (SEAO), with a focus on underreported countries such as Vietnam. Additionally, a geographical analysis was performed to compare disease burden, sequencing coverage, and land use between highly and understudied regions. Sequencing data for SEAO isolates at a high spatial resolution (state/province level) were obtained from the National Center for Biotechnology Information (NCBI). From Vietnam, 48 newly collected B. pseudomallei clinical and environmental isolates were sequenced. Whole genome sequencing was used to assess genetic diversity, with core single nucleotide polymorphisms (SNPs) identified and analyzed to construct a maximum likelihood phylogenetic tree.

Results: Vietnamese isolates were found to be genetically diverse and scattered across multiple clades, suggesting the existence of multiple distinct lineages rather than a single outbreak or clonal expansion. These isolates usually cluster close to Thai isolates,

indicating shared evolutionary histories or regional transmission dynamics. In contrast, Malaysian isolates belong to more cohesive clades, implying a more constrained or clonal population structure. Notably, the isolates from Vietnam and Malaysia do not intermingle on the tree, suggesting they are genetically distinct with limited evidence of recent gene flow between the two regions.

Conclusions: These findings reveal that the separation among these isolates aligns with known ecological and geographic distinctions between mainland and peninsular Southeast Asia, emphasizing the influence of geography on B. pseudomallei population structure.

97. GEOPHYSICAL DRIVERS OF ANTHRAX: A REMOTE SENSING APPROACH TO SOIL TEXTURE CLASSIFICATION TO INFORM OUTBREAK PREDICTION

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Anthrax, a potentially fatal zoonosis among grazing wildlife and livestock, frequently spills over to humans. Endemic near-globally, its causative agent, the sporulating Gram-positive bacterium Bacillus anthracis, persists metabolically dormant in the environment, with outbreaks triggered by host exposure to endospores, often through soil. As anthrax ecology is still not completely understood, geophysical variables are known to improve statistical models predicting the distribution of the pathogen. Evidence suggests soils with higher pH and high organic content are favorable to B. anthracis persistence. While variables

highly correlated with soil texture, such as soil pH, wetness, and vegetation indices, are frequently incorporated into prediction models for anthrax, these models often rely on lower-resolution global datasets that fail to capture landscape heterogeneity reflected by changes in soil texture at short spatial distances. A barrier to integrating soil texture into analysis is the lack of high-resolution gridded soil classification data. This study investigates filling spatial gaps in soil texture classification using Sentinel-2 satellite remote sensed products and Random Forests (RFs) to classify topsoil (depth of 0-30cm) at fine spatial scales (10m gridded resolution) informed by Landsat 8 multispectral product at the lower 30m resolution. While digital soil mapping attained through machine learning methods has been put into practice for precision agriculture, there is limited study on the usefulness of precise soil characterization for predicting pathogen distributions. Our model was trained with legacy data sampled from USDA-NRCS Gridded Soil Survey Geographic (gSSURGO) Database and bioclimactic and remotely sensed data and terrain attributes namely sourced and processed with Google Earth Engine's (GEE) Python API. Model was cross-validated with a 10 k-fold cross-validation strategy and accuracy metrics were assessed with measures including overall accuracy (OA) and the spatial kappa index (K-index). Final analysis employed the Getis-Ord Gi*(d) statistic with categorical dummy variables to assess overlaps between anthrax outbreaks in wildlife populations and interpolated geophysical characteristics on two Texas ranches. Ultimately, this investigation seeks to explore the potential for geophysical classification to serve as an alternative means of predicting geography vulnerable to environmentally-dependent epizootics at small spatial scales, particularly among ranch-bound grazing livestock and localized wildlife populations.

98. HIDDEN CONSEQUENCES: THE SPATIAL OVERLAP OF FLOODING, AGRICULTURE, AND DISEASE IN VIETNAM

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In 2020, Vietnam was ranked the 6th most affected country in the world to climate variability and extreme weather hazards. Flooding was the most prevalent hazard to impact Vietnam in the recent decade and it ranks 1st (alongside Bangladesh) in countries at risk for flooding. These extreme events can create direct and often immediate burdens on communities, as well as indirect downstream consequences. Direct effects of extreme flood events can include injuries and fatalities to both humans and animals, soil and coastal erosion (landslides), and damage across agricultural lands, homes, and built infrastructure. Indirectly, these events can lead to increased exposure to disease (often through contaminated flood waters), food shortages, and widespread economic loss. The intersection of these vulnerabilities can compound risks, disproportionately affecting certain populations. This study examines the spatial distribution of extreme flooding, rice paddy agriculture, and important waterborne diseases in Vietnam to identify environmental intersections that heighten vulnerability. Melioidosis and leptospirosis were selected as exemplar diseases due to their strong associations with flooding and paddy field agriculture. Flood data (1960–2018) were sourced from the Geocoded Disasters Dataset (GDIS), while paddy field distribution was derived from the Global Lakes and Wetlands Database version 2 (GLWD v2). A literature review provided historical disease reports for melioidosis and leptospirosis in humans and animals.

Provincial access to safe drinking water and livestock density (cattle, buffalo, swine, poultry) were calculated using Vietnam Census data (2018–2022). Geographic overlaps were identified using bivariate mapping for both flooding and paddy agriculture as well as livestock and paddy agriculture. Exploratory spatial analysis revealed geographic overlaps of flooding and paddy rice agriculture along the coast and within the southern Mekong Delta. Overlaps between livestock farming and paddy fields were also observed, particularly in the northern Red River Delta. These findings highlight regions where communities may face compounding risks. Expanding disease reporting networks and integrating government-backed monitoring programs could improve public health and resilience, particularly for agricultural workers in flood-prone areas.

99. HUMAN SPOROBIOTA MEDIATES COLONIZATION RESISTANCE AGAINST CLOSTRIDIOIDES DIFFICILE INFECTION IN A GERM-FREE MOUSE MODEL

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Clostridioides difficile is the leading cause of antibiotic-associated nosocomial infections. Fecal microbiota transplantation (FMT) is an effective treatment for patients with recurrent C. difficile infection (rCDI), but the specific microbiome species and their functions contributing to its high efficacy remain poorly understood. Increasing evidence suggests that gut sporobiota may mediate resistance against C. difficile (CD). These sporobiota are members of the Firmicutes phylum, and can survive ethanol treatment. We previously identified 8 unrelated healthy human donors whose fecal microbiome were resistant to C. difficile infection and colonization when transferred to germ-free C57BL/6 mice. The present study aims to determine the efficacy of the ethanol-resistant fraction (i.e. sporobiota) of the 8 human microbiota in C. difficile colonization. Groups of otherwise

identical germ-free (GF) C57BL/6 mice were orally gavaged with fecal suspensions from these 8 donors that had been pre-treated with ethanol. Three weeks post colonization, mice were challenged with C. difficile VPI 10463 spores. C. difficile growth, toxin production, and microbiome composition were analyzed. Of the 8 fecal microbiomes examined, 3 (37.5%) of the sporobiota retained resistance against C. difficile colonization when gavaged into GF mice (i.e. mice were asymptomatic, no CD burden or toxins in cecum), but 5 (62.5%) lost their colonization resistance (i.e. mice were asymptomatic but had detectable CD burden and toxins in cecum). Microbiome analysis identified specific taxa that were differentially abundant between the two phenotypes. These results suggest interindividual differences in sporobiota resistance against C. difficile, which may partially explain the differences in FMT efficacy in clinical settings.

100. IMPACT OF BIOLOGICAL SOIL AMENDMENTS OF ANIMAL ORIGIN, INCLUDING HEAT-TREATED POULTRY PELLETS AND SEABIRD GUANO ON THE SOIL MICROBIOME IN CALIFORNIA.

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Introduction: Biological soil amendments of animal origin (BSAAO) are commonly used as organic fertilizers to enhance soil fertility, but their application carries the risk of introducing foodborne pathogens into agricultural soils. This study aimed to investigate the effects of BSAAO on soil microbial diversity, community structure, and the proliferation of pathogenic bacteria within the soil microbiome.

Methods: A 42-day experiment was conducted in California to evaluate the soil microbiome amended with heat-treated poultry pellets (HTPP) and seabird guano (Guano). Raised-bed plots were left unamended (UA) or amended with HTPP or seabird guano. Soil samples were collected on days 0, 1, 7, 14, 21, 28, 35, and 42. Bacterial genomic DNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina Miseq platform. Data were analyzed with the QIIME2 pipeline. Differences in Shannon and Chao1 indices and microbial relative abundance were analyzed using ANOVA, while differences in Bray-Curtis distances were analyzed using PERMANOVA.

Results: HTPP-treated soils consistently exhibited significantly lower Shannon and Chao1 indices across all sampling days compared to other treatments. Additionally, the bacterial community composition of HTPP-treated soil was significantly distinct from those of the Guano and UA treatment groups. Microbial relative abundance analysis showed that HTPP treatment significantly increased populations of the Proteobacteria phylum compared to other treatments throughout the experimental period. At the genus level, Acinetobacter dominated in the HTPP-treated group, whereas Nicrososphaeraceae became dominant in Guano-treated and UA soils. Opportunistic pathogens, including Psychrobacter, Glutamicibacter, and Massilia, were more

abundant in both HTPP- and Guano-treated soils compared to UA soils.

Conclusions: These findings suggested that BSAAO amendments may increase the risk of soil contamination by promoting populations of pathogenic bacteria.

101. INVESTIGATION OF A VIABLE BUT NON-CULTURABLE STATE IN PORPHYROMONAS GINGIVALIS AND HOST CELL INVASION

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Porphyromonas gingivalis (P. gingivalis) is a gram-negative, black-pigmented, anaerobic pathogen known for its biofilm formation and its central role in periodontal disease. More recently, P. gingivalis has been implicated in various systemic conditions, including atherosclerosis, Alzheimer's disease, and certain types of cancer, such as pancreatic and oral cancer. This bacterium employs several mechanisms to evade environmental stress, contributing to its pathogenicity. The viable but non-culturable (VBNC) state is characterized by bacteria which remain viable but have reduced metabolic activity and are unable to form colonies on conventional culture media. To induce the VBNC state in P. gingivalis strain W83, we subjected the bacteria to oxidative stress using H2O2 and subsequently attempted to resuscitate them from

this state with sodium pyruvate. We utilized viability staining, confocal microscopy, and flow cytometry for counts of live and dead bacteria to confirm the presence of significant numbers of viable P. gingivalis cells both before and after stress induction and resuscitation. Despite their viability, the stressed P. gingivalis failed to form colonies on blood agar plates after seven days, indicating they had entered the VBNC state. We were then able to resuscitate the VBNC bacteria by the addition of sodium pyruvate, as the growth of these cultures on plates was comparable to that of the untreated culture. Investigation into the invasiveness of P. gingivalis in the VBNC state was conducted using human coronary artery endothelial cells. P. gingivalis in the VBNC state demonstrated the ability to invade the cells, with a significant portion of the bacteria persisting within the host cells for extended periods. In this study, we explore the VBNC state in P. gingivalis, a survival strategy previously described in many aerobic bacteria but not in anaerobic bacteria. The objectives of this study were to verify the VBNC state in P. gingivalis, explore whether this state can be reversed, and assess the extent to which this state impacts P. gingivalis ability to invade host cells. Understanding VBNC-related mechanisms will be instrumental in guiding the development of more effective therapies for periodontitis and other associated diseases with P. gingivalis infection.

102. METABOLIC ADAPTATION OF STAPHYLOCOCCUS AUREUS IN SIMULATED MICROGRAVITY

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Introduction: The opportunistic pathogen Staphylococcus aureus was previously isolated from astronauts' nostrils upon return from spaceflight missions. S. aureus has a repertoire of metabolic pathways and this metabolic versatility is important for virulence. Multi-omics comparisons of flight (FLT) and ground control (GC) cultures from the previous “BRIC-23” experiment conducted on the international place station revealed increased AGR quorum sensing and altered expression of virulence factor and metabolic genes in FLT samples. However, it remains unclear how S. aureus metabolically adapts to growth in simulated microgravity (SμG), and whether growth temperature influences this adaptation.

Methods: S. aureus UAMS-1 was cultured in normal gravity (1 g) and SμG conditions using the same media, temperature (25°C), and growth time (48 hours) used for BRIC-23. Cell pellets (n=4 biological replicates per growth condition) were collected, stored at –80oC, and subjected to RNA isolation and RNA-Seq. CLC genomics workbench software was used for processing RNA-seq data and conducting differential expression analysis. Various web-based bioinformatics tools were also used to analyze transcriptome data.

Results/Discussion: 166 (86 increased, 80 decreased) differentially expressed (DE) genes (SμG/1 g) were obtained after fold-change (≥

2) and statistical (T-test, $p < 0.05$) cutoffs were applied. A strong increase in expression of genes involved in pyrimidine and purine biosynthesis was observed, which may provide the cells with an energy efficient metabolism under environmental stress. Venn analysis comparison to the previously published BRIC-23 FLT/GC RNA-Seq data revealed that 14 differentially expressed genes were common to both the SμG/1 g and BRIC-23 FLT/GC DE data. Examples of the overlapping genes included increased expression of the quorum-sensing system gene agrD (2.9- and 14.8-fold increase in SμG/1 g and FLT/GC, respectively). Increased expression of stress resistance genes (otc, SAR1921, narT, scdA) was also observed in the SμG/1 g DE data, suggesting that these cultures may be experiencing stress-induced cellular damage.

103. MODELING ANTHRAX RISK AT HIGH RESOLUTION USING WILDLIFE CASE DATA AND LANDSAT COVARIATES FOR THE TEXAS ANTHRAX TRIANGLE

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Introduction: Predicting the geographic distribution of pathogens is important for developing targeted surveillance and disease control. For pathogens with environmental reservoirs, ecological niche models (ENMs) predict locations of persistence. For anthrax,

caused by the bacterium Bacillus anthracis, pathogen persistence can last years or decades in certain environments. Typically, ENMs use moderate or low-resolution covariates, often with MODIS satellite data (250- or 1-km) and/or interpolated ground station data (1-km or larger pixels). Across large areas, such as national or regional scales, these models provide a useful first pass to determine passive surveillance areas. However, higher resolution models, using 30-m Landsat data can provide better estimates for smaller areas, such as individual ranches or wildlife management areas/national parks, where anthrax needs to be managed on the ground. Often, such models are restricted by availability of data for deriving presence points and computing time.

Methods: Toward this, we curate a ~25-year record of specific carcass locations of more than 200 unique locations and, for nearly half of those, we know the specific lineage of B. anthracis associated with the cases. Here, we used a combination of time-specific Landsat 8 data and continuous vegetation indices, including NDVI, EVI, SAVI, and the tasseled cap transformation to develop a new 30-m covariate set to model the potential distribution of two major linages in the Texas Anthrax Triangle (TAT) of West Texas with a focus on several specific ranches actively managing the disease in wildlife and livestock.

Results: Models improve with covariant resolution and, more importantly, by genetic lineage, with each Vollum and Ames have distinct geographic distributions, with Ames habitat across the south of the TAT.

Conclusions: These new models provide insights into anthrax landscape ecology and redefine risk areas at a resolution managers can use to fine tune surveillance efforts.

104. MULTIVALENT OUTER MEMBRANE VESICLE (OMV) VACCINE FOR MELIOIDOSIS AND GLANDERS

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The objective of this research project is to develop a multivalent outer-membrane vesicle (OMV) vaccine targeting melioidosis and glanders, severe infectious diseases caused by Burkholderia pseudomallei (Bp) and B. mallei (Bm), respectively. These pathogens are classified as Tier-1 select agents due to their potential to be used in a bioterrorism event, and their resistance to antibiotic therapy. Despite previous efforts, there are currently no effective vaccines against these biothreat agents. Recently, the Centers for Disease Control and Prevention (CDC) declared Bp to be endemic in the state of Mississippi based on melioidosis reported in three patients with no travel history to an endemic country. Soil sampling in the immediate vicinity of these individuals revealed clonal populations of pathogenic Bp. Environmental modeling suggests that the entire Gulf Coast of the United States may be conducive to the growth of Bp in the soil and is an emerging threat in the United States. These pathogens have the ability to evade the host immune system, leading to acute pulmonary infection and sepsis, with chronic infection and relapse being common in melioidosis. Most vaccines developed so far have failed to provide long-term protection, particularly against different serotypes or genotypes of Bp. We hypothesize that OMVs derived from the less pathogenic related bacteria B. thailandensis (Bt) and biosafe attenuated Bp strains can offer better protection against melioidosis and glanders compared to those from a single

Bp strain. We also suspect that OMV antigens from Bt may be less immunosuppressive than those from pathogenic Bp. This research project strives to design a multivalent OMV vaccine containing different O-antigen types to enhance protection and reduce regulatory T-cell responses. The study will involve in vitro, in vivo, and ex vivo experiments to identify OMV composition, assess protection in mice, study T cell responses, assess for cellular toxicity of the OMV, and design vaccination strategies. The goal is to develop OMV vaccines that provide broad protection against diverse strains and serotypes of Bp and Bm, ultimately achieving sterilizing immunity and long-term protection against these biothreat agents.

105. PATHOGENS ASSOCIATED WITH MORTALITY OF FARMED WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN FLORIDA, 2016-2024

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The farming of white-tailed deer (Odocoileus virginianus) is a growing and economically consequential industry in Florida, and infectious diseases are the greatest threat to the health of animals and commercial success of farmers. The Cervidae Health Research Initiative provides free necropsies and diagnostics for diseases afflicting farmed deer in Florida. This study sought to identify the suite of pathogens associated with mortality in captive deer and the dynamics of the diseases they cause, with particular attention to disease seasonality and age group susceptibility. A total of 918 white-tailed deer that died between 2016 and 2024 were included in this analysis. Of those animals, 879 (95.8%) were tested for hemorrhagic viruses, of which 178 (20.3%) tested positive for bluetongue virus (BTV) and 252 (28.7%) for epizootic hemorrhagic disease virus (EHDV). There was strong seasonality in BTV and EHDV case load concurrent with typical BTV and EHDV insect vector densities, with case load peaking in the fall. Diagnostic bacteriological cultures performed on selected tissues from the necropsied animals revealed involvement of bacteria in 589 animals (64.2%). The most common bacterial isolates were Escherichia coli (n = 300), Trueperella pyogenes (n = 177), Streptococcus species (n = 110), and Pseudomonas species (n = 85). Bacterial infection case load also peaked in the fall, but the seasonal increase appeared to begin slightly before the increase in

hemorrhagic disease cases. There were 235 cases (25.6%) of coinfections involving both hemorrhagic virus and bacteria. Case characteristics also varied according to age group, with hemorrhagic viruses afflicting a larger proportion of juveniles (n = 196, 68.5%) than fawns (n = 67, 22.3%) and adults (n = 83, 25.0%), and bacterial infections afflicting a slightly larger proportion of fawns (n = 204, 68%) and adults (n = 24, 64.5%) than juveniles (n = 59.8%). This pattern is likely explained by host immunological and both host and vector ecological factors. A stronger understanding of the diversity of pathogens present in captive deer populations, and the effect of such co-infections, is crucial to developing adaptive management strategies that safeguard farmers from economic loss, improve captive animal welfare, and promote the maintenance of healthy wild animal populations.

106. POTENTIAL BIOCONTROL AGENTS IN THE MICROBIOME OF RED IMPORTED FIRE ANTS AROUND THE EMERGING PATHOGENS INSTITUTE

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This study explores bacterial communities associated with the Red Imported Fire Ant (RIFA), Solenopsis invicta, to identify bacteria that could be used for biocontrol applications. From 3 fire ant nests located near the Emerging Pathogens Institute Building at the University of Florida, Gainesville, FL, bacterial strains were isolated from the external and internal surfaces of ants, nest soils,

and soils located 5 meters from their nests. We used a total of 4 different selective media, including: nutrient agar, Luria-Bertani (LB) agar, tryptic soy agar, and chitin agar. A total of 468 bacteria strains were isolated, their DNA was extracted, and 127 16S rRNA gene sequences were analyzed. The identified genera included Acinetobacter, Bacillus, Chryseobacterium, Comamonas, Cronobacter, Enterobacter, Klebsiella, Lysinibacillus, Massilia, Microbacterium, Paenibacillus, Pantoea, Pseudomonas, Serratia, and Xanthomonas. Most isolates belonged to the Bacillaceae family, with Bacillus being the most prominent genus (89 isolates). The core microbiome of RIFA nests was dominated by Bacillaceae and Pseudomonadaceae families, with chitin agar supporting the growth of bacteria not observed in other media, including Comamonas sp. and Variovorax sp. Certain bacteria, such as Proteus penneri and Microbacterium hydrocarbonoxydans, were found exclusively in ant eggs, larvae, and pupae, suggesting potential ecological roles within the nest environment. Several identified bacteria, including Pseudomonas sp., Serratia sp., and Bacillus sp., are known insect pathogens and some have been used in biocontrol strategies. These findings align with previous research on microbial communities in ants and hint at the possible entomopathogenic properties of some isolates. Future research will continue to explore these bacteria for their use in biocontrol applications, potentially offering a novel approach to managing fire ant populations.

107. PREVOTELLA CORPORIS ACTIVATES A PROTECTIVE HOST STRESS RESPONSE THAT PREVENTS AND REVERSES TOXIC PROTEIN AGGREGATION

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Neurodegenerative protein conformational diseases (PCDs) such as Alzheimer's, Parkinson's, and Huntington's disease are characterized by the buildup of protein aggregates that lead to neuronal death. The protein homeostasis, or proteostasis network, is orchestrated by mechanisms that collectively maintain proteome stability. The proteostasis network ultimately addresses protein aggregation and disaggregation. Recently, we have demonstrated that colonizing the Caenorhabditis elegans gut with bacteria associated with dysbiosis leads to protein aggregation across distal tissues, suggesting bacteria may play a role in maintaining organismal proteostasis. In a comprehensive screen of 229 bacterial strains, we identified the genus Prevotella as a robust suppressor of protein aggregation in C. elegans models of PCDs. Prevotella is associated with a healthy human microbiota, yet the mechanism underlying the observed proteoprotection is unknown. To elucidate the mechanisms through which these bacteria exert a proteoprotective effect on the host, we employed strains of C. elegans expressing hsp70::GFP, cytoplasmic heat shock response reporter, and PolyQ::YFP, a sensor for protein stability. We discovered that of the 229 species, P. corporis, not only induces the expression of hsp70—a critical chaperone involved in maintaining cellular proteostasis—but also reverses the buildup of polyQ aggregates in older worms. Furthermore, we employed RNAi gene knockdown to demonstrate that HSF-1 is required for the observed

P. corporis-mediated induction of the Hsp70, confirming the involvement of the canonical HSR. The transcription factor HSF-1 is well known for its role in protein folding and disaggregation; however, its activation by bacteria was not observed before. Here, we characterize the bacteria-mediated regulation of protective stress responses, enhancing host proteostasis, and galvanize new microbial approaches for treating PCDs.

108. RESISTANCE-DRIVEN OUTBREAK DETECTION AND ETIOLOGIC INVESTIGATION FOR HOSPITAL-ONSET PSEUDOMONAS AERUGINOSA USING MACHINE LEARNING ON ELECTRONIC HEALTH RECORDS

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Introduction: Hospital outbreak identification often lacks standardization and relies on time-intensive surveillance methods, which may delay intervention against multidrug-resistant hospital-

acquired pathogens. To address this challenge, we demonstrate combining space-time permutation analysis with machine learning to statistically identify hospital-onset Pseudomonas aeruginosa outbreaks and potential outbreak-specific etiologies contributing to the spread.

Methods: We retrospectively analyzed hospital-onset P. aeruginosa isolates collected between 2016 and 2024 from electronic health records (EHR) at a single tertiary care center. Hospital-onset infections were defined as culture-positive samples obtained more than two days after admission. Antibiotic susceptibility profiles were standardized by imputing for intrinsic resistance and classifying multidrug-resistance (MDR) and extensively drug-resistance (XDR). Space-time permutation analysis (WHONET-SatScan) identified clusters of resistance profiles, with cluster confirmation performed against whole-genome sequencing (WGS) data using SNP differences. For each cluster, we conducted case-control studies comparing affected hospital-onset cases with non-case P. aeruginosa patients hospitalized during the same period. Feature selection for potential temporally-scaled hospital-based risk factors was performed using elastic net regularization (R package glmnet) with 10-fold cross-validation.

Results: Between 2016 and 2024, 19,055 hospitalizations (11,112 patients) yielded culture-positive P. aeruginosa isolates, with 12,498 (65.6%) community-onset and 6,557 (34.4%) hospital-onset cases. Hospital-onset isolates were 1.9 (95% CI: 1.8–2.1) times more likely to be MDR and 2.2 (95% CI: 1.9–2.6) times more likely to be XDR compared to community-onset cases. Of the hospital-onset isolates, 615 unique resistance profiles were identified, with 53% fully susceptible to major antipseudomonal classes. Resistance to antipseudomonal cephalosporins was most common (30.2%; n=1,981), with 27% resistant to cefepime and 27.6% to ceftazidime.

Space-time permutation analysis detected 12 unique clusters, though none overlapped with clusters identified via WGS. One cluster defined by cephalosporin resistance (n=17) was further examined. Final elastic net model identified 32 features associated with cluster membership compared to controls (n=213), including an open excision procedure 5–9 days prior (OR: 28.1, p<0.001) and preparation for skin graft 2 days prior (OR: 45.2, p<0.001).

Discussion: We demonstrate the use of EHRs to statistically detect related hospital-onset P. aeruginosa infections and potential etiologies associated with these clusters. Several factors with strength in association and biological plausibility were identified, though resistance profile-based models did not capture relatedness among clusters confirmed by WGS. Future research will establish causal links and extend this framework to other hospital-acquired pathogens.

109. TARGETING THE GUT PATHOBIOME TO REDUCE THE BURDEN OF PROTEOTOXIC BACTERIA

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Human gut microbiota is a complex community affecting health and disease. Gut dysbiosis has been associated with age-dependent neurodegenerative protein conformational diseases (PCDs), including Alzheimer's and Parkinson's diseases. Using Caenorhabditis elegans that express various tissue-specific PCD-associated proteins and sensors of protein folding, we screened 229

isolates from the Human Microbiome Project for bacterial species that affect protein aggregation upon intestinal colonization. Among the detrimental proteotoxic bacteria, we found Shigella spp. which consistently and significantly induced toxic protein aggregation in the host. Furthermore, recent studies revealed that Shigella reduces the abundance of butyrogenic bacteria, which are known to be protective against many ailments, including PCDs. While Shigella is most often associated with shigellosis, a gastro-intestinal infection, it can also colonize individuals asymptomatically. Their detrimental effect on the protective butyrogenic microbes, combined with the ability to disrupt protein folding and induce toxic aggregation, emphasizes the need to eradicate these microbes from the human gut. Antibiotics are used as a control method for targeting bacteria; however, they also disrupt the protective commensal microbiota and enrich for proteotoxic antibiotic-resistant strains. In this study, we explored the potential of bacteriophage (phage) therapy as a targeted and sustainable solution for combating Shigella-induced proteotoxicity. We isolated four unique Shigella phages (vB-UF/SF1-4) with high specificity and broad host range. Further characterization revealed their robust temperature and pH stability and exceptional specificity and efficacy in killing Shigella flexenri and Shigella sonnei strains, making them ideal candidates for phage-mediated targeting of proteotoxic bacteria. The morphological analysis of the isolated phages using the Transmission Electron Microscope (TEM), revealed that our phages belong to Siphoviridae and Podoviridae families. To enhance the lytic infection kinetics of the isolated phages, we formulated a phage cocktail, which demonstrated superior killing efficacy compared to individual phages. Additionally, we combined the phages with silver nanoparticles (AgNPs), which exhibit a minimum inhibitory concentration (MIC) of 3.1 μg/mL against S. flexneri). The results

revealed that when lower than the MIC of Ag-NPs was combined with a very low multiplicity of infection (MOI) of phages including MOI 0.001 and MOI 0.00001, the mixture completely inhibited S. flexneri growth for more than 14 hours. Based on these results, our approach can specifically eliminate detrimental bacteria, potentially restore gut eubiosis, and affect the pathogenesis of PCDs.

110. UNDERSTANDING DOXY-PEP USE, SIDE EFFECTS, AND MEDICATION SELF-EFFICACY AMONG GAY AND BISEXUAL MEN WHO HAVE SEX WITH MEN (GBMSM): A MIXED-METHODS STUDY OF BARRIERS, BEHAVIORS, AND PROVIDER SUPPORT.

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Background: Doxycycline post-exposure prophylaxis (Doxy-PEP) has emerged as a promising strategy to reduce bacterial sexually transmitted infections (STIs) among gay, bisexual, and other men who have sex with men (GBMSM). However, limited evidence exists on real-world use, side effects, and factors influencing medication self-efficacy and adherence.

Methods: This mixed-methods, cross-sectional study, part of the 2024 PrEPared Study, explored medication self-efficacy, Doxy-PEP side effects, and provider/medication-related barriers among GBMSM. Fifty-eight cisgender GBMSM (23 Doxy-PEP users, 35 non-users) were recruited from the AMETHST cohort. Online surveys collected data on demographics, health behavior, healthcare access, Doxy-PEP awareness, and SEAMS scores. Bivariate analyses

and linear regression assessed associations between Doxy-PEP use and medication self-efficacy. Semi-structured interviews examined side effects, provider guidance, and symptom management strategies. Interviews were transcribed verbatim and reviewed for quality. Qualitative analysis followed a four-phase coding process to identify key themes.

Results: Doxy-PEP users had statistically significant higher SEAMS scores (median = 40) than non-users (median = 37; p = 0.0017), indicating greater self-efficacy in medication use. Most Doxy-PEP users reported mild side effects with nausea (34.7%) and diarrhea (13.04%) being most common. Qualitative findings corroborated these results, revealing that most users alleviated side effects through food intake and confirmed nausea as a frequent experience. Regarding provider counseling, 60.86% (n = 14) received guidance on when to take Doxy-PEP, and 43.47% (n = 10) were told to take it as prescribed. These patterns were consistent across both quantitative and qualitative data. Doxy-PEP use was also statistically significantly associated with PrEP use, chemsex participation, higher risk for substance misuse (CAGE scores), and greater number of male sexual partners.

Conclusion: Doxy-PEP users reported higher medication self-efficacy with only mild side effects. However, comprehensive provider guidance is essential to minimize these effects and raise awareness among users. As Doxy-PEP is a relatively new intervention, further research and real-world evidence are needed to better understand its impact and support its broader use for STI prevention.

111. UNRAVELING THE MICROBIAL GUARDIANS: EXPLORING CRUCIAL BACTERIAL SPECIES DIVERSITY PROTECTING MICE FROM CLOSTRIDIOIDES DIFFICILE INFECTION

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The commensal gut microbiota plays a crucial role in safeguarding the host against enteric pathogens, including Clostridioides difficile (C. difficile), a devastating healthcare-associated infection. Mouse models have been extensively employed to investigate the gut microbiota. Since conventional mice are generally resistant to C. difficile infection (CDI), C. difficile challenge in these animals requires pre-treatment with antibiotic cocktails. Germ-free (GF) mice colonized with murine Firmicutes are resistant to C. difficile challenge. In the present study, we examined the association between Firmicutes diversity and richness and the degree of phenotypic resistance against C. difficile challenge. C57BL/6 germ-free mice were orally gavaged with increasing dilutions (1:5, 1:10, 1:20, 1:40) of murine Firmicutes, thereby generating groups of

mice with varying degrees of microbiome diversity and richness. Mice harboring lower dilutions were asymptomatic and survived the challenge, but remained colonized with C. difficile with detectable toxins (i.e. carrier mice). In contrast, mice colonized with higher dilutions of Firmicutes succumbed to CDI (i.e. susceptible mice). C. difficile toxin levels at the endpoint (14 days post-infection) were significantly lower in the carrier mice compared to susceptible mice. 16S rRNA sequencing analysis identified eight taxa that were differentially abundant between the two groups. Furthermore, metabolomics analysis revealed the enrichment of seven bile acids in resistant mice, suggesting a potential mechanism by which these bile acids contribute to the protective effect against CDI. Rescue of carrier mice with murine Firmicutes consortium significantly reduced C. difficile burden and toxins, but surprisingly, failed to eliminate C. difficile from colonization These results demonstrate that Firmicutes diversity and richness correlate strongly with phenotypic C. difficile resistance, and suggest that an adequate number and diversity of Firmicutes is required for resistance against CDI.

112. VIBRIOSIS IN THE EASTERN UNITED STATES: ENVIRONMENTAL DRIVERS AND IMPLICATIONS OF CLIMATE CHANGE

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Background: Vibrio spp. are ecologically significant bacteria that thrive in warm, moderately saline water, and their incidence and proliferation are heavily influenced by environmental factors. Vibriosis, an infection caused by ca. twelve Vibrio spp., has been reported more frequently in the eastern US over the last few decades, coinciding with alterations of the aquatic environment related to climate change. Here we have analysis of vibriosis case presence in the eastern US connected with environmental parameters associated with climate change.

Methods: Latitudinal distribution trends of six Vibrio spp. vibriosis cases recorded in the CDC COVIS database for 1990 – 2019 along the eastern US coastline were analyzed. Vibriosis case presence and absence were modeled using environmental data with extreme gradient boosting (XGBoost) models. Environmental data related to vibriosis case presence was clustered using k-means clustering.

Results: The northernmost latitude of the reported vibriosis cases was found to have increased approximately 42.2 km per year, with

some species exhibiting more movement than others. Average accuracies of XGBoost models were between 60.9-71.0 %, with models showing SST and SSS as the variables of highest importance. Phytoplankton and precipitation helped differentiate models, but relationships were often nonlinear. K-means clustering results showed three distinct combinations of SST, SSS, and chl-a values for all Vibrio spp., indicating environmental patterns of infections.

Conclusion: The results of this study indicate that environmental parameters associated with climate change are likely drivers of vibriosis case presence. Additional investigation is in progress to assess environmental parameters and vibriosis incidence in other geographic areas, with the goal of developing global predictive models for vibriosis.

113. VIRULENCE DIVERSITY AMONG DIFFERENT P. GINGIVALIS STRAINS

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Background: Porphyromonas gingivalis (P. gingivalis) plays a crucial role in chronic periodontitis and has been implicated in systemic diseases such as cardiovascular disease, rheumatoid arthritis, certain cancers, Alzheimer's disease, and preterm birth. The pathogenicity of P. gingivalis is highly strain-dependent, with significant genetic diversity influencing virulence, biofilm formation, and host interactions. This study aimed to assess strain-specific variations in P. gingivalis invasion and persistence in human coronary artery endothelial cells (HCAECs) and differences in biofilm formation and virulence using the Galleria mellonella model.

Methods: HCAECs were infected with different P. gingivalis strains at an MOI of 100, and bacterial invasion and persistence were quantified via CFU counts at 2.5, 24, 48, and 72 hours post-infection. Confocal microscopy was used to assess intracellular localization. Biofilm formation was analyzed in a 96-well plate model using safranin staining and optical density measurements. Virulence was evaluated in G. mellonella larvae injected with 10^6–10^8 CFU of P. gingivalis, with survival rates and hemocyte concentrations recorded over 72 hours.

Results: Strain W83 exhibited the highest invasion ability, followed by 1102-2-1, FO566, and FO568, while A7A1-28 and AJW4 showed minimal invasion. Persistence in HCAECs varied, with viable bacteria recovered from FO566, FO568, W83, and UF1102-2-1 at 48 hours but none at 72 hours. Biofilm production showed significant inter-strain variability. In G. mellonella, all strains were

pathogenic, with higher virulence correlating with lower larval survival and reduced hemocyte concentrations at 3 hours post-infection.

Conclusion: P. gingivalis strains exhibit considerable virulence diversity, affecting invasion, persistence, biofilm formation, and host-pathogen interactions. These findings highlight the need for strain-specific approaches in diagnostics and treatment strategies, paving the way for precision medicine applications in periodontal and systemic disease management.

114. A RAPID CRISPR-BASED SELF-TESTING PLATFORM FOR EARLY DETECTION OF HIV

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According to the World Health Organization (WHO), an estimated 39.9 million people were living with Human Immunodeficiency Virus (HIV) at the end of 2023, including 1.2 million in the United States. Acute HIV infection, the earliest stage of HIV, typically develops within 2-4 weeks post-exposure, during which the virus rapidly multiplies and spreads. Therefore, early detection is critical to improving individual health and lowering transmission rates. The Centers for Disease Control and Prevention states that HIV can be detected as early as 10 days post-infection using nucleic acids tests, compared to 18 days for antibody-based tests. Although there are several commercially available nucleic acid tests for HIV approved by the Federal Drug Administration (FDA), they are high-complexity lab-based tests requiring expensive reagents and equipment increasing cost and limiting accessibility for widespread screening. The only FDA-approved in-home HIV test, OraQuick, detects HIV through an antibody-based test. However,

there are currently no FDA-approved nucleic acid-based point-of-care or self-testing kits for HIV detection.

This project aims to develop a non-invasive, rapid, simple, and specific self-testing kit using clustered regularly interspaced short palindromic repeats (CRISPR) technology. CRISPR-based diagnostics, coupled with microfluidic platforms, offer great potential for point-of-care testing. Our approach involves extracting HIV-1 RNA from whole blood (WB), serum, or plasma paired with RT-LAMP (reverse transcription loop-mediated isothermal amplification) and CRISPR-based detection, which generates a fluorescence signal for diagnosis within a microfluidic device. Currently, our work focuses on improving lysis techniques to extract HIV-1 RNA from HIV-WB patient samples and further engineering CRISPR to improve our overall sensitivity and specificity of detection. We ultimately seek to deliver a robust strategy to enhance the efficiency and accuracy of our rapid HIV diagnosis platform to serve as a global HIV detection solution.

115. ALCOHOL REDUCTION IS ASSOCIATED WITH LOWER LBP AND SCD14 AT 2-YEAR FOLLOW-UP IN PEOPLE WITH HIV

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Background: Chronic inflammation in people with HIV (PWH) is associated with morbidity and mortality. Heavy drinking is common among PWH and likely contributes to inflammation. Consequently, alcohol reduction may normalize inflammatory markers in PWH. However, few studies have evaluated associations between reduction in drinking and inflammation among PWH, with mixed findings. Associations may be confounded by concurrent changes in mental health, other substance use (e.g., cannabis or tobacco use), or viral load. The present analysis examines alcohol change-plasma biomarker associations while adjusting for time-varying confounding.

Methods: PWH were recruited in Florida (N = 91, contributing 212 person-visits; 37% with baseline heavy drinking) for a 2-year prospective observational study. At yearly visits, alcohol use (light/heavy/none) was categorized using the AUDIT-C. Alcohol increase and decrease at 2-year follow-up (vs. baseline) were operationalized as ≥50% change in AUDIT-C. Past-month cannabis use days (non-daily/daily/none) were categorized with the Timeline Follow-Back. Plasma sCD14, sCD163, lipopolysaccharide-binding protein (LBP), and TNF-α were quantified using the Luminex FLEXMAP bead-based immunoassay. Longitudinal linear random intercept models were fit for each biomarker. Initial models fit fixed effects for time (0, 1, 2) only, representing group-level change across the full sample. Follow-up models included baseline alcohol use (light/heavy/none), AUDIT-C change group (increase/decrease/stable), and alcohol change-by-time interaction. Fully-adjusted models included age, sex, and time-varying depressive symptoms, cannabis use, smoking, and detectable viral load.

Results: Among participants with baseline heavy drinking, 29% reduced drinking at 2-year follow-up; among those with baseline light drinking, 52% reduced drinking and 26% increased drinking. In initial longitudinal models, LBP increased over time (β = 0.33, 95% CI = 0.19, 0.48); no other biomarker showed a main effect of time. In models including alcohol use, sCD14 (β = -0.24, 95% CI = -0.47, -0.01, p = 0.039) was lower in participants who reduced drinking (vs. stable drinkers), adjusting for level of baseline drinking. In fully-adjusted models, associations with sCD14 were substantively similar (β = -0.27, 95% CI = -0.52, -0.02, p = 0.034); in adjusted models only, LBP showed a similar (albeit statistically nonsignificant) effect magnitude (β = -0.22, 95% CI = -0.44, 0.00, p = 0.053). Alcohol-by-time interaction was not associated with TNF-α or sCD163.

Conclusions: Alcohol reduction among PWH is associated with lower levels of inflammatory markers relevant to microbial translocation at follow-up. The clinical relevance of these findings warrants further investigation.

116. DEEP DOWN IN FLORIDA: SAMPLE CHARACTERISTICS OF ADULTS WITH HIV IN WAVE 4 OF THE FLORIDA COHORT – A DESCRIPTIVE STUDY

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Introduction: HIV remains a public health issue. In the United States, Florida is a high prevalence and high incidence setting, with 128,497 Floridians living with HIV and over 4,725 new diagnoses in 2023. Given these trends in Florida, we will fail to meet the Ending the HIV Epidemic (EHE) goal by 2030. Historically, sociodemographic factors contribute to HIV risk. The goal of the

Florida Cohort (FC) is to have a representative sample of adults who are receiving HIV care in Florida to identify barriers and assess how individual, clinic, and community level factors influence steps in the HIV Care Continuum. Therefore, the purpose of this study is to describe the sample characteristics of the FC Wave 4 compared to the state's surveillance data.

Methods: The FC Wave 4 enrolls adults with HIV (AWH) from clinics and case management agencies in three regions across the state. Data are obtained from a questionnaire and linked to the state's surveillance data, which will be compared to sample characteristics. Recruitment began in June 2024 and is ongoing, with 368 of a planned 1,000 AWH enrolled. Sample characteristics of FC Wave 4 were compared to the Florida Department of Health (FDoH) surveillance data from AWH in Florida.

Results: Of the 368 AWH, 62% were >50 years old with a mean age of 52 (SD ± 13) compared to 56% AWH being >50 years old in the FDoH surveillance data. Participants were: 50% Non-Hispanic Black, 25% Non-Hispanic White, 20% Hispanic, and 5% Other compared to 42%, 27%, 27%, and 2% among the surveillance data. Fifty-seven percent of the sample identified as male and 42% identified as female compared to 74% and 25% at the state level. The majority (57%) identified as heterosexual, 29% as homosexual, and 14% as bisexual/other. Surveillance data indicated, however, 55% of individuals acquired HIV through male-to-male sexual contact (MMSC), while 37% reported HIV acquisition through heterosexual contact. Regionally, 32% of participants were from North Florida, 46% were from Central Florida, and 22% were from South Florida. Among all AWH in Florida, 14% reside in North Florida, 33% reside in Central Florida, and 53% reside in South Florida.

Conclusion: The current sample is not representative of AWH in Florida in terms of age, race/ethnicity, sexual orientation, and region. To achieve the EHE goals, future recruitment efforts should focus on obtaining a more diverse sample of participants who are younger, Hispanic, homosexual males, and reside in South Florida.

117. DEVELOPMENT OF PASSIVE VIRAL EDNA DETECTION METHODS FOR MULE DEERPOX VIRUS

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Florida is home of numerous emerging pathogens especially in livestock. These new emerging infections are often associated with high morbidity, mortality, and economic loss. Florida deer farms are at a special risk from these pathogens due to the lack of research and logistical constraints of dealing with cervids such as white-tailed deer (Odocoileus virginianus). Of particular concern, Mule Deerpox Virus (DPV) is regularly found on Florida deer farms and displays a high mortality in fawns up to 3 months. While

recently characterized, little about the disease including transmission is unknown and current detection methods are unreliable. To address these research gaps, we first developed a quantitative PCR assay to detect DPV. Second, we addressed the role of insects in the transmission of the virus. And third, we developed a passive method of DPV in the environment by testing environmental DNA (eDNA) in reservoir samples. Other mammalian poxviruses typically transmit mechanically through vectors. With this information, we collected 195 Muscid flies and 389 Tabanus flies from three Florida white-tailed deer farms and tested them for DPV DNA to understand preliminary vector competency, and 70 soil samples and 38 fecal samples to understand environmental transmission. Current preliminary results demonstrate that rather than mechanical transmission, DPV seem to persist in the population and in the environment. These neoteric methods will allow us to diversify our detection of viral pathogens using non-invasive techniques. With this broader understanding of the environment's role in disease transmission we will be able to identify disease presence faster. A greater understanding of poxvirus transmission is extremely important to protect the health of both farmed and wild cervids.

118. DO NOT HAVE NIGHTMARES ABOUT NOROVIRUS: WASH THEM AWAY

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This research project was developed to inform populations of proper hand washing technique with soap is the best way to prevent illnesses from noroviruses. Norovirus is mainly transferred through the fomite route and can cause issues with the gastrointestinal tract such as acute gastroenteritis. This poses a momentous problem to international public health and the economy. This research observed the use of hand dryers and paper towels, and their effectiveness in lowering pathogen transmission. A study compared public areas within an airport disinfection routine. While another observed the emerging technologies to increase the adherence of hand washing and manual processes. This research assignment is to differentiate hand washing from antiseptic hand wash, hand rub, and surgical hand antisepsis.

This research convey simple hand friction under water with soap is more preventative than the use of hand sanitizer. According to the CDC, individuals are to wash their hands for 20 seconds under warm water with the use of soap. Instructing and demonstrating to patients and persons on techniques of hand washing is necessary. Patients are instructed to wet their hands with warm water prior to applying soap to their hands. Lathering up the entire hand (anterior and posterior) and wrist with the soap. To scrub the palm of the hand, nails, and the back of the hand for at least 20 seconds. Rinsing off the hands with warm water and using a towel to turn off the faucet and using another towel to dry their hands. Manual processes such as posters and placement of sinks in certain institutions encourage hand washing and decreasing the spread of

norovirus and other pathogens. As well as the use real-time location systems (RTLS) that disclose the use of an employee's badge and their location to provide more direct information on the use of restrooms and break rooms. One study compared 30 volunteers use of automatic hand dryers and paper towels usage after hand washing to aid in the reduction of norovirus on the hands.

This research project noted the significance of proper hand washing is the most effective for decreasing the presence of norovirus.

This research project reveals that there are many routes to use to reduce the presence of norovirus on the hands, but proper hand washing is the best defense.

119. EVOLUTIONARY HISTORY OF A NOVEL RECOMBINANT CORONAVIRUS CIRCULATING IN SHREWS IN HLAWGA NATIONAL PARK, MYANMAR

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Monitoring the emergence of novel zoonotic pathogens in wildlife is crucial to prevent spillover into humans, especially in conflict-stricken countries such as Myanmar. We report the identification of a novel recombinant coronavirus (MMAR0293 CoV) in C. fuliginosa shrew in Hlawga National Park, Myanmar that is closely related to Wénchéng shrew virus (WESV) circulating in China. A total of six shrew samples collected in 2017 were sequenced using a shotgun metagenomic approach. De novo assembly was performed with MEGAHIT and compared to NCBI nt database using BLASTN and BLASTX. From MMAR0293 sample we obtained eight contigs that were closely related to WESV (NC_035191.1; nt percent identity 81% – 94%). These contigs add to a partial CoV genome 6,608 bp long. Multiple alignments were obtained using MAFFT based on single MMAR0293 CoV contigs built with WESV genomes obtained from GenBank. Recombination analysis was conducted in RDP5 before maximum likelihood (ML) tree construction with IQ-TREE. RDP5 showed evidence of multiple recombination events in WESV isolates KY967723.1, KY967724.1, KY967725.1, KY967726.1, and KY967734.1, which was corroborated by phylogenies. There was no indication by RDP5 that our novel MMAR0293 CoV is a recombinant with Chinese WESV isolates. The multiple intraspecies recombination evidence suggests that WESV circulates extensively in China in large reservoir populations allowing for multiple recombination events; similar results may be apparent in Myanmar if further sampling was conducted there. Our findings not only expand the known geographic distribution of CoVs to include Myanmar, but also provide additional support for the role of shrews in CoV evolution, persistence, and transmission dynamics in Asia and a possible mechanism for CoV spillover into new mammalian hosts.

120. HOST RNA-BINDING PROTEINS ELAVL4 AND PABPC4L SUPPORT EPSTEIN-BARR VIRUS LYTIC REPLICATION

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Epstein Barr Virus (EBV), a gammaherpesvirus, is causally linked to several cancers, including Burkitt lymphoma (BL), and yet, EBV-targeted therapies do not exist. The lytic phase of EBV plays a significant role in the development of EBV-related cancers; however, activation of this cycle occurs only in a subset of latently-infected cells, complicating the identification of gene expression changes unique to lytic versus refractory populations within bulk cultures. This study aimed to identify cellular transcripts exclusively expressed in the lytic subpopulation of BL cells and to assess their contribution to the lytic cycle. By analyzing differentially expressed transcripts in sorted lytic and refractory HH514-16 BL cells, we discovered that transcripts for the RNA-binding proteins ELAVL4 (HuD) and PABPC4L were significantly enriched in cells undergoing lytic reactivation; these proteins are known to stabilize transcripts. We confirmed that both ELAVL4 and PABPC4L were transcriptionally upregulated in response to lytic triggers and following the expression of the EBV lytic switch gene BZLF1 in HH514-16 cells. Depletion of ELAVL4 and PABPC4L

significantly impaired viral genome replication and reduced virus release. Our investigation into the underlying mechanisms revealed that this depletion led to decreased levels of thymidine kinase transcripts, while the loss of PABPC4L also diminished the abundance of viral DNA polymerase transcripts. This indicates that ELAVL4 and PABPC4L play crucial roles by contributing to the function of the viral DNA replication machinery, supporting the lytic phase of EBV. Interestingly, we observed that the depletion of these proteins also resulted in the upregulation of several viral lytic transcripts—an unexpected outcome that suggests a previously unrecognized role in suppressing transcription. This suppression may help prevent transcription-replication collisions on the viral genome, further contributing to EBV replication.

121. INVESTIGATING PHYLOGENETIC RELATIONSHIPS OF HSP70H PROTEINS ENCODED BY VIRUSES IN THE FAMILY CLOSTEROVIRIDAE

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Closteroviridae is a family of positive-strand RNA plant viruses, containing economically impactful viruses on a global scale, such as citrus tristeza virus. One distinguishing feature of the members in this virus family is the presence of a gene encoding a homolog of cellular 70-kilodalton heat shock proteins (HSP70h). Previous research has defined the function of HSP70h as a necessary movement protein that interacts with cellular myosin filaments

and localizes virions to the plasmodesmata for cell-to-cell movement. Viruses are known as gene robbers, suggesting that a homolog closely related to cellular HSP70s was likely acquired from a cellular host and integrated into a virus genome. Cellular HSP70s are conserved through evolution for virtually all organisms, making this protein a good candidate for phylogenetic analysis. Previous phylogenetic analysis of the Closteroviridae HSP70h proteins identified that HSP70h sequence similarity is correlated with the virus genus and the corresponding insect vector, however, the cellular origin of HSP70h proteins remains unknown. With increased availability of sequence data, we aim to: 1) conduct phylogenetic analysis of the Closteroviridae HSP70h amino acid sequences to determine the most likely viral and cellular ancestors of HSP70h proteins and 2) identify potential structural changes of these homologues over the course of evolution. An alignment of the amino acid sequences of the HSP70h proteins encoded in 78 genomes of the members of the Closteroviridae family was assembled, and a phylogenetic tree was constructed using this alignment, identifying blueberry virus A as the oldest common ancestor to the other HSP70h proteins. The HSP70h amino acid sequences were then scanned through the PFAM database to identify established domains of these proteins. The separated domain sequences were then aligned to construct phylogenetic trees for each domain to observe if a particular domain has an evolutionary pattern differing from the full length HSP70h sequences. Alphafold3 predictions were generated to highlight potential structural differences between the HSP70h proteins analyzed in this study. In future studies, we aim to use blueberry virus A as the root of a phylogenetic tree comparing cellular HSP70 amino acid sequences to further explore potential origin of HSP70h in Closteroviridae. We also will utilize Alphafold3 to predict potential interactions between Closteroviridae HSP70hs

and host myosin proteins to predict if the virus movement could be host specific. The results from this analysis and future studies could provide increased insight into how viruses acquire genes from cellular hosts over the course of evolution.

122. IS PSYCHOSOCIAL DISTRESS ASSOCIATED WITH ANTIRETROVIRAL THERAPY ADHERENCE? CROSS-SECTIONAL DATA FROM MEN WHO HAVE SEX WITH MEN LIVING WITH HIV IN FLORIDA

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Introduction: Men who have sex with men (MSM) are disproportionately affected by HIV. Viral suppression is key for improving individual health and reducing transmission, but requires adherence to antiretroviral therapy (ART). Suboptimal ART adherence is significantly associated with unaddressed social determinants of health. Psychosocial distress is an indicator of unmet social needs. Therefore, this study examines whether

psychosocial distress is associated with ART adherence among MSM in Florida.

Method: The Florida Cohort Wave III enrolls adults with HIV who are receiving HIV care in Florida from eight clinics and case management agencies around the state to help identify barriers to assess how individual, clinic, and community level factors influence steps in the HIV Care Continuum. Cross-sectional data from the Florida Cohort collected from January 2022 through November 2023 were analyzed. Level of psychosocial distress in the past 7 days was assessed using the National Comprehensive Cancer Network's Distress Thermometer, a visual analogue scale where scores range from 0 (no distress) to 10 (extreme distress) ART adherence was operationalized as yes (taking ART as prescribed on ≥85% of days in the past 30 days) or no (taking <85%). Analyses were conducted in SAS using logistic regression. Variables were selected based on a priori knowledge and statistical significance where variables that were associated with the outcome in the unadjusted were included. Race/ethnicity, employment status, household income, housing status were variables selected for the final adjusted model.

Results: Of the 201 MSM were included in the analytic sample, 46% were aged 50+, 32% identified as non-Hispanic Black/African American, and 19% identified as Hispanic or Latine. The median psychosocial distress score was 4 (IQR,1-7). Eleven percent of participants had suboptimal ART adherence. In the unadjusted model, MSM who self-reported higher psychosocial distress were less likely to adhere to ART (OR 0.85, 95%Cl, 0.73-0.98) compared to MSM with lower psychosocial distress. In the adjusted model, psychosocial distress was not statistically associated with ART adherence (aOR, 0.88, 95%Cl, 0.72-1.07).

Conclusions: Psychosocial distress was not associated with ART adherence in this sample of MSM. Most men in this sample were adherent to ART, so future studies should seek to enroll more out of care MSM to better study this potential relationship. Future studies should seek to recruit and retain larger samples of MSM from diverse backgrounds to determine if psychosocial distress is correlated with clinical outcomes, such as viral suppression.

123. PANCREATIC STELLATE CELLS AS A NOVEL HIV RESERVOIR

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People with HIV (PWH) are living longer due to the effectiveness of anti-retroviral therapy. Although viremia can be managed, PWH are at greater risk of developing comorbidities including metabolic syndrome and diabetes. The pancreas is a critical endocrine organ, and little is known about HIV in the pancreas. Using RNASeq data through the Human Pancreas Analysis Program (HPAP), computational analysis was performed to detect the expression of HIV receptors (CD4, CXCR4 and CCR5) in 200,000 cells isolated from human islets. This analysis identified small subsets of cells expressing both CD4 and CXCR4. Further analysis revealed expression of both CD4 and CXCR4 in immune, endothelial and stellate cells. Stellate cells are a rare cell type that is understudied but can be found surrounding pancreatic ducts, acini and blood vessels. Immunohistochemistry of human pancreatic stellate cells

in culture confirmed the expression of CD4 and CXCR4 but also unexpectedly CCR5. Further evidence of HIV receptor expression was gathered using flow cytometry and digital PCR. Stellate cells were then evaluated for HIV permissivity by infection with HIV strains (IIB, JR-FL 89.6) and immunostained for p24 at 3- and 6-days post infection. The results showed productive infection of stellate cells by HIV. Ongoing experiments will inform on cell inflammatory status, the rate of viral replication and viral latency. To our knowledge, these are the first findings that implicate stellate cells as a possible reservoir for HIV in the pancreas with implications for endocrine dysfunction in PWH.

124. REPTARENAVIRAL PCR SCREENING OF PERUVIAN HERPETOFAUNA

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Reptarenaviruses genus within the Arenaviridae family, are enveloped, segmented, negative-stranded RNA viruses known to cause Boid Inclusion Body Disease (BIBD), a fatal neurological disorder in captive snakes. While historically associated with captive populations, recent studies have identified Reptarenavirus in wild snakes, suggesting a broader host range and geographic distribution. In 2022, the virus was documented in free-ranging Boa constrictor in Costa Rica, raising questions about its presence in other South American populations and a possible co-evolution with this snakes it their natural habitad. In Peru 28 oral swabs were collected from boid snakes including both captive and free-ranging origin individuals. The sampled snakes consisted of native species and illegally imported individuals confiscated by Peruvian

authorities. RNA extraction was performed using the Qiagen RNeasy kit, followed by PCR amplification and electrophoresis. PCR products were transported to the United States for sequencing, avoiding regulatory barriers associated with unprocessed nucleic acids. Sequence analysis identified 14 positive cases in captive boas and 2 in free-ranging boas, with the detected strains showing genetic identity to the University of Giessen Reptarenavirus, a well-documented BIBD-associated virus.This represents the first report of Reptarenavirus in Peruvian boas and the second in free-ranging snakes worldwide. The presence of a known BIBD-associated virus in both captive and wild populations can be assosiated with long-term host-virus coevolution. These findings highlight the need for increased disease surveillance, expanded sampling of wild populations, and the development of in-country diagnostic capacity to support wildlife health and conservation efforts in Peru and beyond.

125. TECHNOLOGY-BASED INTERVENTION FOR ALCOHOL AND HIV: DESCRIPTION OF AN ONGOING PROJECT

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Background: Antiretroviral therapy (ART) adherence is key to maintaining HIV viral suppression, but many people with HIV (PWH) face barriers to adherence, such as alcohol use. In general, there are few effective alcohol interventions for PWH, and alcohol interventions for PWH often lack the potential for integration into HIV care systems. Thus, a more integrated approach is needed to incorporate interventions into clinical, community, and research settings to increase effectiveness of care and synergy with other services.

Method: 80 adult PWH who meet the NIAAA heavy drinking criteria will participate in a one-month observational study where they complete daily ecological momentary assessment (EMA) surveys, wear a BACtrack Skyn wrist alcohol sensor. Some participants will also use the alcohol sensor in conjunction with PL Cares, an app designed to connect PWH to healthcare, and complete interviews assessing interest and feasibility of a combined intervention.

Using the alcohol wrist sensor, we will collect transdermal alcohol concentration (TAC) readings for objective and continuous monitoring of drinking behaviors . We will also obtain daily smartphone-based EMA survey data through the MetricWire app, to collect information on topics such as alcohol consumption, other drug use, the participant's environment, and adherence to antiretroviral medications.

TAC data from the alcohol sensor will be charted using TASMAC, an Excel macro, which will allow us to examine several patterns of alcohol consumption, including individual days or drinking episodes.

Each participant will also be asked to provide two dry blood spot samples at day 14 and 28 using the HemaSpot HF kit from SpotOnSciences. This data will allow us to examine HIV viral load in real time together with alcohol use and other factors .

Results: As we have currently only enrolled 3 of our 80 participants, the study is still a work in progress.

TAC data from recently completed participants will be presented.

Conclusion: With various remote data collection technologies, data from this project will allow us to assess barriers to ART adherence and consequentially HIV viral suppression in participants' typical day-to-day environment with a more fine-grained time scale. Additionally, feedback from those who complete the PL Cares integration segment will inform future alcohol interventions that consider the specific needs of PWH with the potential for integrating the alcohol biosensor into an existing HIV care app. This study will also provide information on the practicality and reliability of HemaSpot HF kits, a novel method for examining HIV viral load.

126. VALIDATION OF A ONE-POT RT-LAMP CRISPR/CAS12B PLATFORM FOR RAPID DETECTION OF TILAPIA LAKE VIRUS

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Tilapia lake virus (TiLV) is a pathogen causing significant morbidity and mortality in both farmed and wild tilapia globally. Many diagnostic tools for TiLV have been developed, however, they require complex equipment and lengthy processes, making them impractical for resource-limited laboratories or pondside use. In order to address this challenge, we developed and validated a novel, rapid, and cost-effective one-pot diagnostic test that integrates a thermostable Cas12b enzyme with RT-LAMP amplification, targeting a conserved region within segment 4 of the virus genome. This assay is user-friendly, as it needs only an incubation step at 62°C for 75 minutes, with results observable through a portable fluorescence viewer. The TiLV one-pot assay is both sensitive and specific, detecting as few as 50 RNA viral copies and showing no cross-reactivity with other fish RNA and DNA viruses. Furthermore, despite primer mismatches, it successfully identified 12 TiLV transcripts from different geographic regions. Evaluation of 151 positive samples and 110 negative samples from

challenge studies, field outbreaks, and surveillance, resulted in the diagnostic sensitivity and specificity of 92% and 100%, respectively. Finally, this study presents reports on the validation of a rapid diagnostic assay for TiLV in stages 1 and 2, following WOAH guidelines. Ultimately, this assay can act as a valuable asset in global surveillance, enabling fish health professionals to efficiently monitor and control TiLV disease in tilapia farming.

127. WHITE SPOT SYNDROME VIRUS IN IMPORTED BLUE CRAYFISH (PROCAMBARUS ALLENI) FROM THE ORNAMENTAL TRADE

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Introduction: White spot syndrome virus (WSSV) is a globally important virus that infects all decapod crustaceans and causes economically devastating mortality in penaeid shrimp. While international viral spread has been linked to the trade of crustaceans destined for aquaculture and commodity use, there are only a few reports of WSSV in live crayfish within the ornamental trade. The global ornamental trade is a poorly regulated industry and is considered a major route of invasive crayfish species introduction in parts of Europe. Here, we describe and characterize a WSSV mortality event in imported blue crayfish (Procambarus alleni) from the ornamental trade.

Method: Twenty blue crayfish were purchased from a New York vendor, which had been imported from Thailand. During quarantine, 16 crayfish were serially found dead, five of which were submitted fixed for postmortem examination, and one of which was frozen at -80°C. The remaining surviving four crayfish were subsequently humanely euthanized and submitted fixed for postmortem examination. All fixed crayfish (9/9) were processed for histologic examination, with routine staining. Representative sections from crayfish with and without inclusions were subjected to WSSV in situ hybridization (ISH, 3/9). Pooled samples of fresh-frozen cuticular epithelium, gills, and stomach were subjected to WSSV real-time PCR (qPCR) and Illumina Novaseq next-generation sequencing (NGS) with subsequent phylogenetic analysis.

Results: Histologic examination revealed large intranuclear viral inclusions within tissues of ectodermal and mesodermal origin in all naturally deceased crayfish and two euthanized crayfish (7/9). Those without viral inclusions had mild hemocyte infiltration of similar tissues (2/9). WSSV was confirmed by ISH and qPCR, and NGS generated a genetically distinct 281,051 bp WSSV genome that formed a unique branch among clustered shrimp and prawn isolates from China and Bangladesh, respectively. Importantly, our isolated was evolutionarily distinct from WSSV in other crayfish samples, including the circulating USA isolate.

Conclusions: Given the evolutionary distance of our isolate from that currently circulating within the USA, these crayfish were likely exposed to WSSV within the ornamental trade, be it at the Thailand aquaculture facility, during transit, or at the vendor holding facility. This report exemplifies the poorly documented risk of disease spread through the ornamental trade. Given the propensity for crayfish escape/release in the ornamental industry, the risk of disease spread to wild populations and, to a lesser extent, cultured

crustaceans should be considered when establishing future trade regulations in the ornamental trade.

128. WHOLE GENOME CHARACTERIZATION OF TORQUE TENO SUS VIRUS 1 (TTSUV1) IN WILD AND DOMESTIC PIGS: INSIGHTS INTO GENETIC CLASSIFICATION, HOST DIFFERENTIATION, AND INTRA-HOST VARIATION

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Background: Torque teno sus virus 1 (TTSuV1), a member of the Anelloviridae family, is highly prevalent in swine populations and exhibits substantial genetic diversity. Despite its ubiquity, TTSuV1 remains understudied, particularly regarding its genetic diversity,

host-specific differentiation, and intra-host variation. These characteristics are critical for understanding its evolution, transmission dynamics, and potential applications in biosecurity monitoring.

Methods: Field and laboratory protocols included capturing wild pigs, collecting whole blood samples, and screening for TTSuV1-positive samples through PCR. TOPO TA cloning was used to amplify individual viral variants within hosts, and whole genome sequencing (WGS) was performed on selected clones. A dated phylogenetic tree was reconstructed using TTSuV1 whole genome sequences obtained from wild pig samples in this study and all available sequences from NCBI. To evaluate genetic differentiation between wild and domestic pigs, partial viral sequences (~700 bp) were analyzed using phylogenetic D statistic and analysis of molecular variance (AMOVA). Intra-host variation was assessed by calculating pairwise identity percentages among viral clones from individual hosts and constructing haplotype networks.

Results: Phylogenetic analysis of whole genome sequences grouped TTSuV1 into four clades, with sequences from wild pigs distributed across all clades. Known subtypes 1a, 1b, and 1c were localized within Clades 3 and 4, leaving sequences in Clades 1 and 2 with unidentified subtypes. Partial sequence analysis revealed significant host-specific genetic differentiation: the D statistic confirmed a non-random association between host type (wild vs. domestic) and phylogeny, and AMOVA further showed contributions of both host type and geography to overall variation. Intra-host variation analysis provided evidence for multiple sources of genetic diversity within individual hosts. Pairwise identity percentages among viral clones ranged from 63.6% to 100%, with lower identity values indicating co-infection with distinct viral variants. Haplotype network analysis revealed

mutational steps between haplotypes from the same host, suggesting that intra-host evolution also contributes to within-host genetic variation.

Conclusions: This study highlights the significant genetic diversity and host-specific differentiation of TTSuV1, with wild pigs playing a key role in its evolution. Both intra-host evolution and co-infection contribute to its diversity, underscoring its potential as a tool for monitoring biosecurity risks and cross-transmission between wild and domestic pigs.

129. ACCURATE AND NON-INVASIVE BIOFILM MONITORING IN DRINKING WATER DISTRIBUTION SYSTEMS THROUGH EFFLUENT ANALYSIS OF QUORUM SENSING-RELATED MRNA LEVELS

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Biofilm formation in drinking water distribution systems (DWDS) poses risks to water quality and public health. As bacteria attach to the inner walls of pipes, they can form complex structures known as biofilm, in which bacteria are shielded from external disturbances like antibiotics and disinfection. Upon growing to a certain size, biofilms can shed cells into the water, leading to an increase in bacteria in the distribution system that residual disinfection may not account for. This shedding can expose individuals to pathogens, making it crucial to monitor their levels from the tap; however, traditional sampling methods are impractical, requiring direct access to pipe surfaces that can require special appurtenances or increased cost.

This study introduces a novel method for determining biofilm growth in DWDS by correlating it with mRNA levels associated with quorum sensing (QS) systems in tap water. Bacteria communicate using QS systems, which rely on bacterial density to activate the expression of certain genes. The Las QS system is associated with

biofilm formation and growth; therefore, we hypothesize that the dispersal of sessile biofilm cells in DWDS affects the QS-related mRNA levels found in tap water.

We used Pseudomonas aeruginosa PAO1, a common bacteria found in DWDS, as the model organism. P. aeruginosa uses the Las QS system to form biofilms. We focused on analyzing the expression of the lasI gene, a major component of the Las QS system, because it creates the signaling molecule 3OC12-HSL. In the batch experiment, P. aeruginosa displayed significantly higher lasI mRNA levels in biofilms than in planktonic states. Increases in mRNA levels in biofilms preceded an increase in biofilm cells, representing the growth of biofilm following lasI expression. The mRNA levels in the culture media also increased after the rise in mRNA levels in biofilms, suggesting the detachment of biofilm cells that increased the mRNA levels of the planktonic cells. We also cultivated P. aeruginosa biofilms in plastic tubing and flushed it with running tap water to simulate conditions in pipes. The tubing experiment demonstrated that the lasI mRNA levels in the effluent from the tubing correlate with biofilm growth conditions.

Our findings indicate the potential for improved biofilm monitoring, which will advance the management of water quality and protect public health.

130. ASSESSING PRESENCE OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN THE INDIAN RIVER LAGOON: A BAYESIAN APPROACH TO UNDERSTANDING THE IMPACT OF ENVIRONMENTAL STRESSORS

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants, and their presence in aquatic environments, especially coastal waters, poses significant ecological and human health risks. This study investigates the occurrence and behavior of four PFAS compounds in the Indian River Lagoon, a biodiverse estuarine ecosystem located in Florida USA, by evaluating how ecological and hydroclimatic factors influence PFAS occurrence. A Bayesian Logistic Regression Model (BLRM) was employed to quantify the relationships between environmental stressors such as salinity, precipitation, river discharge, water temperature, and pH, and the presence of these PFAS compounds. The BLRM approach not only estimated the log odds of PFAS presence but also provided posterior estimates and odd ratios, making it a transparent and interpretable model compared to other machine learning techniques. The results indicate that salinity is a significant negative predictor for all PFAS compounds, showing a decrease in PFAS presence with increasing

salinity. Precipitation exhibited a statistically significant positive association with PFBS, PFOA, and PFHxS, whereas river discharge negatively affected PFNA and PFOA. Model diagnostics confirmed BLRM's robustness, with posterior predictive checks showing strong alignment between observed PFAS presence and the model's predictions, validating its accuracy. The study highlights BLRM's advantages in environmental modeling, identifying key stressors and the direction of their effects on PFAS occurrence. It emphasizes the importance of ecological and hydroclimatic factors, such as salinity, precipitation, and river discharge, in understanding PFAS behavior in coastal ecosystems. These insights aid future risk assessments and management strategies to mitigate PFAS contamination in aquatic environments.

131. DEVELOPMENTAL AND BEHAVIORAL EFFECTS OF PERFLUOROTRIDECANOIC ACID IN ZEBRAFISH EMBRYOS AND LARVAE

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Perfluorotridecanoic acid (PFTrDA) is a long-chain per- and polyfluoroalkyl substance (PFAS) commonly used in industrial applications such as fluorosurfactants and Teflon production. Despite increasing regulatory attention on PFAS, there remains limited data on PFTrDA's developmental and neurobehavioral toxicity. This study aimed to evaluate the morphological,

behavioral, and apoptotic effects of PFTrDA exposure in zebrafish (Danio rerio) embryos and larvae to help fill this data gap.

Zebrafish embryos at 6 hours post-fertilization were exposed to embryo rearing medium (ERM) with no chemicals, dimethyl sulfoxide (DMSO), or varying concentrations of PFTrDA (0.1–1000 µg/L). Assessments included hatch rate, mortality, and deformities using an EVOS™ FL Auto Imaging System. Locomotor and anxiety-like behaviors were measured using a light-dark preference test via DanioVision™ Observation Chamber, while apoptosis was analyzed using acridine orange staining.

Exposure to PFTrDA resulted in reduced survival across all treated groups, with the highest concentrations showing the greatest mortality. Morphological deformities were infrequent (<1%) but present. Behavioral analysis revealed altered locomotor activity, particularly during the light phase of the light-dark test, suggesting possible neurotoxicity, although the pattern was not consistently dose-dependent. Apoptosis levels were elevated at the lowest tested PFTrDA concentration (0.1 µg/L), with no significant differences observed at higher doses.

These findings indicate that PFTrDA exposure, even at environmentally relevant levels, can induce both lethal and sublethal effects in developing zebrafish. While acute toxicity appeared low to moderate, behavioral and apoptotic changes suggest potential neurological and cellular impacts. Future studies will explore mitochondrial function and gene expression to further elucidate PFTrDA's mechanisms of toxicity.

132. DISCOVERY OF NATURAL REPELLENTS AGAINST THE LONE STAR TICK, AMBLYOMMA AMERICANUM

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Tick-borne diseases are an increasing concern, necessitating the discovery of effective repellents to reduce human and animal exposure to the Lone Star tick, Amblyomma americanum. This study evaluates natural compounds, including essential oils fractionated, and their extracted components, using spatial and fingertip repellent assays. In spatial assays, amyris oil fraction A2 (0.1µg/cm^2) showed significant repellency at 2 hours, with a 13-fold increase compared to the acetone control. Conversely, fraction A3 exhibited an attractant effect, with a 14-fold decrease in repellency. Sandalwood fractions SW1 (10µg/cm^2) and SW2 (10µg/cm^2) showed 86.7% (± 13.3%) and 60% (± 11.5%) repellency, respectively, both significantly higher than the control. Among oil components, compound 1 demonstrated strong repellency at 0.1µg/cm^2 and 10µg/cm^2, with a 16-fold increase in repellent effect at 10µg/cm^2. Fingertip assays revealed that compound 3 (10µg/cm^2) exhibited 70% (± 12.9%) repellency, while compound 4 (1µg/cm^2) and compound 1 (0.1µg/cm^2 and 10µg/cm^2) showed 47.5% (± 2.5%), 52.5% (± 6.3%), and 55% (± 15%) repellency, respectively. These results highlight the potential of amyris and sandalwood oils, along with their specific fractions and components, as environmentally friendly repellents against Am. americanum nymphs.

133. EFFICACY OF ARTIFICIAL INTELLIGENCE PROGRAM IN DETERMINING THE HEALTH STATUS OF MICE

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Malaria is one of the leading causes of death in Africa with a majority of those deaths being in children caused by severe malaria syndromes such as lung or cerebral malaria. We do not fully understand the disease processes in these syndromes. Mouse models are an important tool to expand our knowledge and find viable solutions. However, it is critical that these models be applied with strict humane endpoints to minimize suffering which can be challenging due to rapid onset of disease. Researchers can achieve this by frequent monitoring of infected mice, but this is labor intensive. To meet this challenge, we created a machine-learning (ML) algorithm to evaluate videography to distinguish infected from uninfected mice. Videos of mice infected with Plasmodium berghei NK65 were inputted into the Vertex AI platform and compared to videos of healthy mice. The resulting algorithm, which evaluated data from 14 consecutive days, was able to identify sick and healthy mice from the test data set with an accuracy rate of 92.6%. These results can be built upon to further develop a model that is capable of monitoring mice in real time and notifying users when a mouse's health has declined below a certain threshold to allow for timely and humane intervention.

134. EVALUATING THE DICHOTOMOUS METABOLIC INTERACTIONS IN HUMAN GLIOBLASTOMA TUMORS USING SPATIAL PROTEOMICS ANALYSIS

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Introduction: Within the glioblastoma (GBM) microenvironment, distinct micro-niches arise from independent cancer cell lineages with unique metabolic needs, classified as Fast Cycling Cells (FCCs) and Slow Cycling Cells (SCCs). FCCs primarily rely on aerobic glycolysis, while treatment-resistant SCCs depend on lipid metabolism.

Methods: This study examines the molecular and spatial heterogeneity of GBM, emphasizing immune interactions and the metabolic interplay between SCCs and immune cells in human patients. High-plex immunofluorescence imaging was performed using the COMET platform to investigate the immune contexture in human GBM tumors. Formalin-fixed, paraffin-embedded (FFPE) biopsy samples were labeled with fluorescent antibodies targeting FaBP7 to identify SCC-enriched (FaBP7-high) versus non-SCC (FaBP7-low) regions. Regions of interest (ROIs) were defined based on FaBP7 expression, with 40 ROIs analyzed across five tumor sections from different patients. Immune cell populations were labeled using markers such as CD31, CD45, CD68, CD11b, CD4, HLA-DR, FoxP3, CD8, Vimentin, and VISTA.

Results/Conclusions: This study reveals a connection between spatial metabolic heterogeneity and immune diversity. SCCs exploit immunosuppressive myeloid-derived cells to support tumor metabolism via lipid transport. Inhibiting lipid transfer disrupts SCC metabolism, remodels the immune microenvironment, delays tumor progression, and improves outcomes. Additionally, sensitivity to lipid-modifying drugs, such

as statins, correlates with the SCC phenotype. These findings highlight the metabolic and immune interactions within GBM and suggest therapeutic potential in targeting lipid metabolism to improve patient outcomes.

135. HBO THERAPY IN PARKINSONIAN MICE

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Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder with no disease-modifying treatments. Preclinical studies have suggested that hyperbaric oxygen (HBO) therapy may offer neuroprotective benefits in models of spinal cord injury and traumatic brain injury. However, there is very little data available in literature on the effect of HBO on PD. We hypothesize that HBO can be useful in PD by attenuating neuroinflammation and reducing α-synuclein protein aggregation.

Methods: Animals were maintained on a 12/12 h light/dark cycle with food and water ad libitum, and all procedures were approved

by UF IACUC. At around 8 weeks of age, α-syn Tg Ala53Thr hemizygous (M83+/–) mice received bilateral intramuscular (biceps femoris) injections of 10 μg of α-syn fibrils or PFFs (disease group) or 5μL of sterile PBS (healthy control group). Treatment group received HBO treatment in a hyperbaric chamber for 1h daily for 8 weeks at 2ATA (PFF + HBO: n = 15), and the control group were placed in chamber under normoxic conditions (1ATA) for 8 weeks (PFF: n = 15). Body condition score, and survival were assessed. Microbiota was analyzed using fecal pellets via 16S bacterial rDNA sequencing methods. Unrestrained and unanesthetized animals were studied in a flow- through whole-body plethysmograph chamber to measure breathing.

Results: We observed differences in body weight at 12 weeks post treatment. We did not observe any difference in survival between groups after 8 weeks of HBO (n=7). In HBO animals, we observed less overall microbiota distortions, based on alpha-diversity measures. HBO prevented PFF-associated distortion in microbiota diversity (Shannon index). We also found a statistically significant increase in Prevotellaceae ($p<0.02$) associated with HBO, potentially associated with reduced levels of inflammatory markers. Interestingly, a reduction in Prevotellaceae has been observed in PD patients. Prevotellaceae can produce SCFAs and have been shown to inversely correlate with symptoms in PD patients.

Conclusions: HBO may be a promising therapeutic strategy for treating PD, resulting in microbiota modifications that might have independent efficacy.

136. IMPACT OF SOCIO-ECONOMIC FACTORS ON MOSQUITO ABUNDANCE AND DIVERSITY

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Income in an area has been shown to significantly correlate with the abundance of anthropogenic mosquitoes such as Aedes aegypti and Culex quinquefasciatus in urban systems. This difference has been attributed to factors such as poor infrastructure (e.g. inadequate waste management, sanitization), housing conditions (e.g. lack of screens on door and windows), and limited resources for mosquito control agencies resulting in a higher abundance of breeding sites. However, limited research has examined mosquito burden in suburban and rural communities. To fill this gap, we assessed mosquito abundance and diversity in 6 low-and high-income neighborhoods in Gainesville, FL. We also conducted a KAP (knowledge, attitude, and practice) survey of residents' understanding of mosquito biology, mosquito-borne disease transmission, and mosquito control techniques. We found the Aedes aegypti abundance was greater in lower-income neighborhoods, and that residents of these neighborhoods reported a greater overall impact of mosquitoes in their daily lives than residents of higher-income neighborhoods. We also found that residents of lower-income neighborhoods were more likely to attempt to conduct practices meant to mediate the impact of mosquitoes, but were less knowledgeable about mosquitoes, mosquito-vectored pathogens, and effective management techniques than residents of higher-income neighborhoods.

137. LACTALBUMIN FROM MILK WHEY POWDER AS A BLOOD-PRODUCT-FREE ARTIFICIAL MOSQUITO DIET

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Many mosquito research projects require the maintenance of a laboratory-reared colony so that researchers have consistent access to high quality, physiologically consistent mosquitoes for laboratory and semi-field scale assays year-round. Most mosquitoes are anautogenous and require a blood meal to reproduce. Thus, mosquito colonies require the use of laboratory animals (mammals or birds) or whole blood (either animal or human) to support continued rearing. Each of these approaches can be challenging and/or resource intensive; therefore, a blood-product-free artificial diet is highly desirable. Previous research has established that albumin is an important and effective fraction of whole blood, which enables, and may be sufficient for, mosquito egg generation. To the best of our knowledge, other groups attempting to replace whole blood or surrogate animal blood feeding involved the use of blood-derived products, such as bovine serum albumin (BSA,) bovine hemoglobin, or sprayed porcine blood, which can be costly, difficult to obtain, or highly perishable under normal laboratory conditions. In this study, we used milk whey powder as an albumin source in place of blood feeding, which contains no blood or blood-derived products. This diet effectively maintains laboratory strain colonies of Aedes aegypti and Anopheles quadrimaculatus for a minimum of 6 (AEG) and 4 (AnQ) generations, with promising potential for it to become an enduring practice. Here, we report data relevant to the mosquitoes' physical characteristics as compared to parallel blood-fed

laboratory colonies. This work highlights the potential of utilizing milk whey powder as a blood meal alternative for the continual rearing of laboratory mosquito colonies.

138. MICROBIAL ALLIES: A MICROBIAL PROTEIN PREVENTS MURINE COLITIS

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Inflammatory bowel disease (IBD) is characterized by gastrointestinal inflammation comprised of Crohn's disease and ulcerative colitis. Centers for Disease Control and Prevention report that 1.3% of the population of the United States (approximately 3 million people) were affected by the disease in 2015, and the number keeps increasing over time. IBD has a multifactorial etiology, from genetic to environmental factors. Most of the IBD treatments revolve around disease management, by reducing the inflammatory signals. We previously identified the surface layer protein A (SlpA) of Lactobacillus acidophilus that possesses anti-inflammatory properties to mitigate murine colitis. Herein, we expressed SlpA in a clinically relevant, food-grade Lactococcus lactis to further investigate and characterize the protective mechanisms of the actions of SlpA. Oral administration of SlpA-expressing L. lactis (R110) mitigated the symptoms of murine colitis. RNAseq analysis revealed an elevation of

tryptophan catabolism in dendritic cells. We further investigated the role of tryptophan metabolites in R110 mediated protection.

139. MICROBIOME DRIVES AGE-DEPENDENT SHIFTS IN BRAIN TRANSCRIPTOMIC PROGRAMS AT THE SINGLE-CELL LEVEL IN DROSOPHILA

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The gut microbiome plays a critical role in brain function and the brain-gut axis, yet its cellular and molecular mechanisms remain unclear. Here, we present the first comprehensive single-cell transcriptomic atlas of brain cells from adult Drosophila melanogaster raised under axenic and conventional conditions, spanning both young and old ages. Profiling 34,427 cells across 101 clusters, we annotated 56 distinct cell types and identified cell-type-specific gene signatures influenced by the microbiome. Transcriptional shifts were most pronounced in aged flies, with glial cells and dopaminergic neurons among the most microbiome-responsive cell types. Notably, glial cells emerged as one of the most affected populations, exhibiting robust transcriptional changes. Differentially expressed genes (DEGs) were enriched in pathways related to mitochondrial activity, energy metabolism, and Notch signaling. Functional validation confirmed that mitochondrial activity is significantly elevated in glial cells under conventional microbiome conditions. We also quantified age-associated changes in the gut microbiome, observing reduced Acetobacter dominance and increased microbial diversity that corresponded with heightened brain transcriptional responses. These findings illuminate the cell-type-specific impacts of the

microbiome on brain gene expression and highlight glial mitochondrial remodeling as a key feature of microbiome-brain interaction, laying the groundwork for understanding the molecular underpinnings of the microbiome-gut-brain axis.

140. PATTERNS AND PROBLEMS: ADDRESSING CYCLES AND ISSUES IN THE TREATMENT, CONTROL, AND PREVENTION OF INFECTIOUS DISEASE EPIDEMICS.

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Global health initiatives are increasingly recognizing that societal responses to epidemics can be as critical as scientific responses. To combat infectious disease outbreaks more effectively, it is essential to understand the social factors associated with these events. Integrating sociological factors with traditional public health policies can lead to the development of programs that not only address outbreaks but also help survivors, their families, and communities cope with the aftermath. However, documenting and disseminating societal responses to illnesses caused by pathogens can be important in addressing fears, angers, and disease spreading behaviors. This approach, in turn, enhances the ability to plan for future outbreaks and emerging pathogens. The project details interviews, publications, and undergraduate classes that explore the cyclical nature of responses to infectious disease epidemics throughout history.

This project includes several approaches to documenting the experiences of infectious disease and teaching about those experiences to help address cycles of epidemics, pandemics, and

growth of antibiotic resistance. Part of the projects features oral history interviews with childhood poliomyelitis survivors to document their experiences before, during, and after the pandemic. Other parts of the project include teaching undergraduate courses on the history of social and individual responses to infectious disease outbreaks, with topics ranging from experiences with Poliovirus to multidrug-resistant strains of Acinetobacter baumannii. Exposure to these societal responses better prepares individuals, institutions, and governments to make more informed decisions and public health policies for future epidemics.

141. RATIONAL DESIGN OF AAV-RH.10 TO IMPROVE LIVER DE-TARGETING

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AAV has established itself as the leading viral vector based gene therapy platform to treat a host of diseases in the clinic. However, prohibitively high doses are required for sufficient transduction in extrahepatic tissue due to the high levels of off-target liver transduction. Unfortunately, even when high doses of AAV are administered, low therapeutic indices are often observed in tissues outside the liver. Therefore, there is a critical need to develop AAVs that exhibit reduced liver targeting while maintaining natural tropisms to effectively target extrahepatic tissues at low, non-toxic doses. AAVHSC16 is a serotype with natural liver de-targeting through the presence of three unique residues that reduce galactose binding. AAVrh10 was modified via rational design techniques to resemble AAVHSC16 (rh10-16) at three residues. Here we present a novel rh10 capsid that minimally transduce the

liver. Further, given the ability of rh10 to cross the BBB, we believe this capsid may represent an ideal candidate for delivering therapeutics to the CNS.

142. RICE FALSE SMUT, AN EMERGING THREAT: GLOBAL RISK ASSESSMENT USING THE R2M PLANT HEALTH TOOLBOX

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Rice is a staple food for over half the world's population. Rice false smut, caused by Ustilaginoidea virens, is an emerging global threat, affected by changes in rice varieties and climate shifts, such as altered rainfall patterns and temperatures. Previously considered a minor disease, false smut now presents significant risks due to its increasing prevalence, leading to yield reductions. There is interest in strengthening global risk assessments and monitoring for the disease.

This study aims to (1) identify candidate locations for surveillance, (2) assess the risk of spread through seed trade systems, and (3) map climate suitability for the pathogen. We used the open-source R2M Plant Health Toolbox to evaluate risk based on cropland connectivity, trade networks and synthesize expert knowledge about the disease in Nepal. In Nepal, our findings indicate that the Terai rice-growing regions maintain strong connectivity with neighboring countries, while Nepal's informal seed trade is highly clustered, elevating epidemic risks.

We also developed a risk map based on environmental parameters during rice vulnerability to false smut. Key factors included temperature, humidity, and precipitation. These analyses can be used as a baseline to enhance rice false smut surveillance and inform targeted interventions. More broadly, use of computational tools like R2M in global risk assessment frameworks can provide a baseline for plant disease forecasting and support proactive management.

143. SO NICE, THEY ATE IT TWICE: PRIMARY AND SECONDARY MORTALITY VIA COPROPHAGY OF BLATTELLA GERMANICA USING BAS 438 UB I

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German cockroaches (Blattella germanica) are a world-wide synanthropic pest organism responsible for triggering asthma in young children, allergies, skin irritation, gastrointestinal problems, and pathogen spread. Pesticide resistance has become a significant concern, as these pests have developed mechanisms to evade control. BAS 438 UB I, a slow-acting insecticide, offers a novel mode of action that utilizes a gel bait formula to pass through the alimentary canal and be present in feces. We investigated primary mortality (direct feeding on BAS 438 UB I) and secondary mortality (coprophagy) using susceptible and resistant cockroaches. Our results show that susceptible cockroaches displayed higher mortality in both naïve and donor populations, while resistant cockroaches showed high mortality in donor populations but reduced mortality in naïve populations compared to susceptible populations. BAS 438 UB I is a competent insecticide for primary and secondary mortality. Notably, resistant cockroaches showed reduced mortality in naïve populations, highlighting the need for further research on higher doses to cause more secondary mortality. Future studies should investigate the optimal dose and delivery method to effectively control German cockroach populations and mitigate resistance.

144. SYNERGISM OF PYRETHROID-DERIVED CHEMICAL TCA WITH THREE SYNTHETIC VARROACIDES

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The Varroa mite (Varroa destructor) is an ectoparasite of honey bees that transmits a variety of pathogenic viruses during feeding. The most common method for controlling Varroa mites is the direct application of synthetic chemicals into beehives. However, the rapid development of resistance to these chemicals has made Varroa mites a major concern for the beekeeping industry. 1R-trans-chrysanthemic acid (TCA), a component of pyrethrin I, has been found to synergize the toxicity and repellency of pyrethroid insecticides. In this study, we aimed to evaluate the toxicity and synergistic effects of TCA when combined with three commonly used varroacides against Varroa mites and honey bees. In contact vial assays, the 24-hour LC_{50} of TCA for Varroa mites was 155 mg/L, indicating that TCA alone is insufficient to be used as a standalone varroacide. Co-treatment with the 24-hour LC_{10} dose of TCA (81.1 mg/L) did not show synergistic effects with coumaphos or amitraz, yielding synergism ratios of 0.71 and 0.74, respectively. However, TCA significantly enhanced the toxicity of fluvalinate, showing a 5.4-fold increase. TCA alone was found to be relatively safe for honey bees, regardless of the exposure method (oral administration or topical application). The transcriptional profiles of detoxification and stress-related marker genes in honey bees remained stable following single or combined treatments of TCA

and fluvalinate, further indicating TCA's safety. Finally, Varroa mites attached to adult worker bees were introduced into Mason jars coated with TCA and fluvalinate, and survivorship was monitored over 48 hours. The co-treatment group showed significantly lower mite survivorship (10%) compared to the TCA-treated (56.7%), fluvalinate-treated (40%), or untreated groups (66.7%). Collectively, these results suggest that TCA is safe for honey bees and exhibits a synergistic effect with fluvalinate against Varroa mites, offering a promising strategy for managing fluvalinate-resistant mite populations.

145. SYNTHESIS AND EVALUATION OF A-RING SUBSTITUTED COMPOUNDS INSPIRED BY MITRAGYNINE WITH AN OPEN D-RING STRUCTURE

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Determining the role of each of the four rings of the indole-based kratom alkaloids, exemplified by mitragynine, on receptor binding proper]es has increased the importance in developing novel compounds. The A ring's structure-actvity relationship (SAR) with opioid receptors (mu, kappa, delta), and alpha adrenergic receptors will be studied by constructing the target molecule from different indole derivatives. Therefore, we aim to assess the impact of removing the D ring on mitragynine's therapeutic effects, binding affinity, and potency. New substituents will be incorporated on the A ring by conducting total synthesis from different methoxylated and chlorinated indole derivatives in order to examine changes in binding affinity and compare to natural

mitragynine with an open D ring (1,2,3,4-tetrahydro-β-carboline). SAR analysis will highlight the specific regions of the molecule crucial for receptor binding. Further research into constructing analogues with open A, B, or C rings will shed light on potential mitragynine pharmacophoric elements.

146. THE ASSOCIATION BETWEEN ANTIDEPRESSANTS AND CURRENT PREP USE MODERATED BY SELF REPORTED MENTAL HEALTH STATUS AMONG SEXUAL MINORITY MEN IN THE U.S.

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Background: In the United States, Sexual minority men (SMM) continue to face a disproportionate burden of HIV. Although pre-exposure prophylaxis (PrEP) significantly reduces the risk of HIV transmission, its uptake remains limited. Antidepressant use may indicate mental health challenges that influence PrEP engagement, yet little is known about how perceived mental health status may shape this relationship.

Methods: This cross-sectional study (36-month assessment) examined the association between antidepressant use and current PrEP uptake among 4,237 HIV-negative cisgender men from the Together 5,000 cohort—a U.S. nationwide HIV prevention study. The primary exposure was past-year antidepressant use (yes/no), and the outcome was current PrEP use (yes/no). Self-reported past-year mental health status was measured using GAD-PHQ scores. Covariates included sociodemographic variables, housing instability, food insecurity, health insurance, PTSD risk, substance use, and PrEP need. Descriptive statistics, bivariate analyses (chi-square), and multivariable logistic regression were performed to evaluate associations and estimate adjusted odds ratios (aORs) with 95% confidence intervals (CIs).

Results: Among participants, 21.74% reported antidepressant use, and 18.79% reported current PrEP use at 36 months. Antidepressant use was significantly associated with higher odds of current PrEP use (aOR = 1.59; 95% CI = 1.32, 1.91; p < .0001). However, participants with high GAD-PHQ scores had lower odds of PrEP use (aOR = 0.76; 95% CI = 0.63, 0.90; p = 0.0017), suggesting that poorer self-perceived mental health may be a barrier despite antidepressant use. Other variables associated with a higher odds of PrEP uptake included higher income, higher educational attainment, health insurance coverage, and use of club drugs such as GHB and ketamine. In contrast, homelessness, lack of insurance, and methamphetamine use were associated with a lower odds of PrEP use.

Conclusion: Antidepressant use is positively associated with current PrEP use among sexual minority men; however, individuals reporting poor mental health despite antidepressant use may remain less likely to engage in PrEP. These findings highlight the importance of integrating mental health screening and support into HIV prevention efforts. Addressing both perceived mental well-being and structural barriers may improve PrEP uptake in this vulnerable population.

147. THE HISTORY OF PUBLIC HEALTH EFFORTS TO COMBAT INFECTIOUS DISEASE IN FLORIDA

Nina Stoyan-Rosenzweig - Department of African Studies, Center for African Studies, University of Florida; **Chris Eaton** - University of Florida; **Alyson Young** - University of Florida

This poster explores change and growth in 20th century Florida public health knowledge and subsequent population health protection and programming through use of a Florida archival collection. This historical document and journal collection began when Jacksonville was the center for the State Board of Health, which was established in 1899 to address epidemics of cholera, yellow fever, typhoid, malaria, hookworm, smallpox and tuberculosis, and the prevalence of a disease called pellagra, originally believed to be infectious, but which was later shown due to a nutritional deficiency. This collection was retained in Jacksonville- even after the center of state public health shifted to Tallahassee- and was held in the library that served multiple hospitals in Jacksonville. Materials continued to accumulate as the libraryremained central to healthcare research as the Jacksonville Hospital Education Program created in 1958. Known familiarly as JHEP, it formed with a collection of hospital libraries who joined together to provide more effective library services to the numerous hospitals in Jacksonville. This group became a central library named after James Borland. The program served by the Borland Library was later renamed the Jacksonville Health Education Program and in 1969 it became a division of the UF Health Science Center. In 1988 the name was changed to UF Health Science Center Jacksonville. Key components of the collection include the publication Florida Health News and this publication in particular is a valuable record of how public health officials received information on new scientific breakthroughs particularly in the

early 20th century. These journals show how beliefs that pellagra was an infectious disease were quickly replaced as research showed it was due to a nutritional deficiency. They also document transformation of ideas about causes of polio, first reflecting a belief that polio was not caused by a pathogen and then reflecting advances in scientific knowledge. Interestingly for a health service based in the American South at the time when there was less trust in the federal authority, Florida officials showed readiness to accept new knowledge and to use that knowledge in developing public health initiatives. These documents also show the history of efforts to create modern and effective outreach efforts and public education programming, and can serve as a valuable resource for researchers interest in learning more about the was scientific knowledge transforms public health outreach.

148. UNDERSTANDING THE IMPACT OF SOIL MICROBIOME ON STRAWBERRY GROWTH AND NUTRITIONAL PROFILES

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Sciences, University of Florida; **Kwangcheol Casey Jeong** - Department of Animal Sciences, Institute of Food and Agricultural Sciences, College of Agricultural and Life Sciences, University of Florida

Background: The rhizosphere microbiome plays an important role in plant growth, nutrient acquisition, and overall health. In this study, we investigated the relationship between the rhizosphere microbiome and the health status of strawberries (Fragaria × ananassa) under identical soil and environmental conditions. Twenty strawberry plants were classified into a well-grown group (H) and a poorly-grown group (UH) based on morphological characteristics, and the soil microbial community was analyzed by 16S rRNA gene sequencing.

Results: The H group had significantly higher nitrogen concentrations, while the UH group had excessive iron, manganese, zinc, and copper accumulations, suggesting a link between microbial composition and nutrient uptake. Microbiome analysis identified Microvirga and JG30-KF-CM45 as the main bacteria associated with plant nutrient status. Microvirga was positively correlated with nitrogen but negatively correlated with micronutrient accumulation, while JG30-KF-CM45 showed the opposite trend. Furthermore, co-occurrence network analysis showed that microbial communities in the UH group exhibited higher levels of competitive interactions, potentially destabilizing the rhizosphere microbiome and impairing plant health.

Conclusion: These findings suggested that microbial interactions within the rhizosphere influence nutrient homeostasis and plant health. The imbalances observed in unhealthy plants emphasize the importance of maintaining a balanced microbial community for optimal crop performance. This study provides valuable insights into the role of rhizosphere microbes in sustainable

strawberry cultivation and emphasizes the potential of microbiome-based strategies to improve plant health and productivity.

149. UNRAVELING IMMUNE MODULATORY PROTEIN INTERACTIONS THROUGH STRUCTURAL MODELING AND COMPUTATIONAL EVOLUTIONARY ANALYSIS

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Suppressor of cytokine signaling 1 (SOCS1) regulates immune responses by inhibiting the JAK-STAT pathway, but its structural interactions remain poorly understood. Recent studies suggest that the SOCS1 dimer binds JAK2 with higher affinity than the monomer, influencing immune signaling and inflammatory diseases. However, structural distortions in the monomer may impact its regulatory function. This study employs AlphaFold-generated models, molecular docking, and the GLM-Score tool to analyze SOCS1-JAK2 interactions across multiple species. Ramachandran analysis assessed structural stability, identifying disallowed regions in the SOCS1 monomer, while GLM-Score predicted receptor-ligand binding affinities to quantify interaction strength. Structural modeling confirmed that the SOCS1 dimer exhibits stronger binding to JAK2 than the monomer. Structural distortions in monomers suggest reduced binding efficiency, potentially altering immune regulation. Comparative analysis identified conserved residues crucial for JAK2 interactions, revealing evolutionary adaptations in SOCS1 function. These

findings provide insight into the molecular basis of SOCS1's regulatory role in immune signaling and its implications for inflammatory and autoimmune disorders. Understanding these interactions may guide future therapeutic strategies targeting the JAK-STAT pathway.

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