Center for Emerging, Zoonotic, and Arthropod-borne Pathogens

2023 CeZAP Infectious Diseases Research Symposium

October 6, 2023

The Inn at Virginia Tech

FRALIN LIFE SCIENCES INSTITUTE
VIRGINIA TECH™

2023 CEZAP INFECTIOUS DISEASES SYMPOSIUM PLANNING COMMITTEE

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Mike Zhang, Professor
Turner Faculty Fellow, Department of Biological Systems Engineering

2023 CEZAP INFECTIOUS DISEASES SYMPOSIUM

7:30 – 8:30 **Check In & Refreshments**
Foyer

Welcome and Opening Remarks
Latham AB

8:30 – 8:45 Kylene Kehn-Hall, Director of CeZAP
Randy Heflin, Senior Associate Vice President, Research and Innovation
Aimée M. Surprenant, Dean of the Graduate School

8:45 – 9:00 **ID-IGEP Program Introduction**
Ann Stevens, ID-IGEP Co-Director
Kevin Edgar, ID-IGEP Co-Director
Jonathan Auguste, ID-IGEP Co-Director

Morning Keynote
Latham AB

"Early warning systems for disease (re)emergence"

9:00 – 9:30 Pejman Rohani, Regents' Professor
University of Georgia

Morning General Session
Latham AB

Session Chairs:
Dana Hawley, Professor, Department of Biological Sciences
Michael Robert, Assistant Professor, Department of Mathematics

9:30 – 9:50 ***"It's a Risk to Handle Money: Networks of Information and Infection During the 1889 Influenza Pandemic"***
Tom Ewing, Professor
Department of History, Virginia Tech

9:50 – 10:10 ***"The Impact of Human Mobility on Covid-19 Dynamics"***
Omar Saucedo, Assistant Professor
Department of Mathematics, Virginia Tech

10:10 – 10:20 ***Break***

10:20 – 10:40 ***"Using Routinely Collected Public Health Data for Epidemiologic Research During the Evolving COVID-19 Pandemic: Challenges, Lessons, And Next Steps in Estimating Vaccine Effectiveness Using Surveillance Data from the Virginia Department of Health"***
Rachel Silverman, Research Scientist
Center for Biostatistics and Health Data Science
Department of Statistics, Virginia Tech

10:40 –11:00 ***"3D Genome Architecture in Individual Development of a Malaria Mosquito"***

Igor Sharakhov, Professor
Department of Entomology, Virginia Tech

11:00 – 11:20 ***"Divergent Evolution of the Typhoid Toxin and Alternative Binding Subunit ArtB was Shaped by the Gain and Loss of Prophage Across Salmonella spp. Lineages"***
Rachel Cheng, Assistant Professor
Department of Food Science and Technology, Virginia Tech

11:20 – 11:40 ***"COVID-19: Agent-based simulation-optimization to vaccine center location vaccine allocation problem"***
Esra Toy, Associate Professor
Grado Department of Industrial and Systems Engineering, Virginia Tech

11:45 – 1:15 *Lunch and Poster Session*
Latham CDEF

11:45 – 12:45 External and Internal Advisory Board Close Door Meeting
Drillfield

Afternoon Keynote
Latham AB
1:20 – 1:50 *"Airborne Disease Transmission from 6 Feet to 6 Million Feet"*
Linsey Marr, Charles P. Lunsford Professor and University Distinguished Professor
Department of Civil & Environmental Engineering, Virginia Tech

2:00 – 3:00 CeZAP Thematic Area Breakout Sessions

Breakout Session #1: Antimicrobial Countermeasures, Pathogen Identification, and Disease Diagnostic
Duckpond
Session Chair: Andrew Lowell, Department of Chemistry, Virginia Tech

2:00 ***"Semisynthetic Strategies to Access Novel Chloramphenicol Derivatives"***
Suzzudul Islam Shuvo, Department of Chemistry, Virginia Tech, ID IGEP affiliated student

2:12 ***"Engineering an Enhanced Auxin-Inducible Degron Degradation System for Rapid Depletion of Fungal Proteins: Expanding Horizons in Antifungal Drug Target Identification and Protein Function Studies"***
Pat Chaisupa, Department of Biological Systems Engineering, Virginia Tech, ID IGEP affiliated student

2:24 ***"Development of novel inhibitors against Venezuelan equine encephalitis virus by targeting capsid-importin interactions"***
Abdullahi Jamiu, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

2:36 ***"Immunogenicity and Replication Capacity of Recombinant Rotaviruses Expressing Norovirus Proteins"***

Charlotte Nyblade, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

2:48 ***"Subspecies-Specific Detection of Xylella fastidiosa with CRISPR-Cas12a"***
Xuemei Missi Zhang, School of Plant and Environmental Sciences, Virginia Tech

Breakout Session #2: Computational Biology and Disease Modeling

Smithfield

Session Chair: Jingqiu Liao, Assistant Professor, Department of Civil and Environmental Engineering, Virginia Tech

2:00 ***"A Synthesis Work on Climate Change and Coastal Ecosystem Health"***
Connor Hughes, Department of Fisheries and Wildlife Conservation, Virginia Tech, ID IGEP affiliated student

2:12 ***"Modeling the Effects of Host Availability and Temperature on Mosquito-borne Parasite Transmission"***
Kyle Dahlin, PhD, Department of Mathematics, Virginia Tech

2:24 ***"ESM_2: A Promising Deep Learning Approach for Identifying Novel Antibiotic Resistance Genes."***
Vineeth Manthapuri, Department of Civil and Environmental Engineering, Virginia Tech

2:36 ***"Ecological Niche Models of Cache Valley Virus; An Emerging Orthobunyavirus in North America"***
John Muller, PhD, Department of Entomology, Virginia Tech

2:48 ***"Mathematical Formulations for Representing Human Risk Response in Epidemic Models"***
Leah LeJeune, PhD, Department of Mathematics, Virginia Tech

Breakout Session #3: Ecology, Epidemiology, and Environmental Microbiology

Cascades

Session Chair: David Popham, Professor, Department of Biological Sciences, Virginia Tech

2:00 ***"Trait-based modelling of predator-prey dynamics in vampire bat rabies endemic countries"***
Shariful Islam, Department of Fish and Wildlife Conservation, Virginia Tech, ID IGEP affiliated student

2:12 ***"Distribution of the Bats of Mali"***
Abdu Alkishe, PhD, Department of Fish and Wildlife Conservation, Virginia Tech

2:24 ***"Ancient Gene Transfers and Co-Evolution Between Eukaryotes and Giant Viruses"***
Sangita Karki, Department of Biological Sciences, Virginia Tech, ID IGEP affiliated student

2:36 ***"The Impact of Brewery Byproducts as a Feed Additive on Farmed Rainbow Trout (Oncorhynchus Mykiss): Spent Yeast (Saccharomyces Cerevisiae) and Black Soldier Fly (Hermetia Illucens) Cultured on Spent Grain"***
Jason Pough II, Department of Food Science and Technology, Virginia Tech, ID IGEP affiliated student

2:48 ***"Entomopoxvirus Associated Polinton-like Viruses Provide Insight into Replicon Evolution"***
Zachary Barth, PhD, Department of Biological Sciences, Virginia Tech

3:00 –3:15 **Break**

3:15 – 4:15 CeZAP Thematic Area Breakout Sessions

Breakout Session #4: Human Dimension of Infectious Diseases, Public Health and Clinical Microbiology

Duckpond

Session Chair: Andrea Bertke, Associate Professor, Department of Population Health Sciences, Virginia Tech

3:15 ***"The Knowledge, Practices and Attitudes associated with Malaria Endemicity in the Tribal Communities of Southeastern Bangladesh"***
Mahjabin Kabir Adrita, Department of Geography, Virginia Tech

3:27 ***"Mortality in Mexico City during the 1890 Influenza Epidemic"***
Sydney Murphy, Department of Biological Sciences, Virginia Tech

3:39 ***"Understanding Impacts from Inflow and Infiltration on Pathogen Detection and Levels in a Rural Sewershed: Toward Universal Application of Wastewater Based Surveillance and Epidemiology"***
Amanda Darling, Department of Civil Engineering, Virginia Tech, ID IGEP affiliated student

3:51 ***"Inhibition of Stress Hormone Receptors Reduces Clinical Recurrences of Herpes Simplex Virus 1 In Guinea Pigs"***
Jillian Green, Department of Population Health Sciences, Virginia Tech, ID IGEP affiliated student

4:03 ***"SARS-CoV-2 Omicron XBB1.5 Shows Altered Replication in Neurons Versus Ancestral WA1/2020, Facilitated By NRP-1"***
Jonathan Joyce, Department of Population Health Sciences, Virginia Tech, ID IGEP affiliated student

Breakout Session #5: Immunology and Host Pathogen Interactions

Smithfield

Session Chair: James Romero-Masters, Assistant Professor, Department of Biomedical Sciences and Pathobiology, Virginia Tech

3:15 ***"Rift Valley fever virus NSs protein interacts with LC3 family members to inhibit antiviral autophagy"***
Kaylee Diana Petraccione, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

3:27 ***"Functional Characterization of a Novel Kinase TGTKL1 in Toxoplasma Pathogenesis"***
Dima Hajj Ali, Department of Biomedical Sciences and Pathobiology, Virginia Tech

3:39 ***"MDM2 Overexpression Reduces Venezuelan Equine Encephalitis Virus Production Independent of its E3 Ligase Activity and the Proteasome"***

Morgen VanderGiessen, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

3:51 ***"Induction of an Endogenous Giant Virus in Chlamydomonas reinhardtii: Friend or Foe?"***
Maria Paula Erazo Garcia, Department of Biomedical Sciences and Pathobiology, Virginia Tech

4:03 ***"Cross Protection Against Usutu Virus and Saint Louis Encephalitis Virus in Mice Treated with West Nile Virus Convalescent Plasma"***
Md Shakhawat Hossain, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

Breakout Session #6: Vector Biology, Vector-borne Diseases, and Zoonotic Diseases

Cascades

Session Chair: Megan Vogt, Post-doctoral Associate, Department of Biomedical Sciences and Pathobiology, Virginia Tech

3:15 ***"Chromosomal Inversions Differentiate Mosquitoes in Culex pipiens Complex"***
Yifan Feng, Department of Entomology, Virginia Tech

3:27 ***"The Role of Culex territans Mosquitoes in the Transmission of Batrachochytrium dendrobatidis to Amphibian Hosts"***
Joanna Reinhold, PhD, Department of Biochemistry, Virginia Tech

3:39 ***"Investigation of the Type-B Muscarinic Acetylcholine Receptor (mAChR-B) as a Potential Insecticide Target for Control of Disease Vectors"***
Brandon Bickley, Department of Entomology, Virginia Tech

3:51 ***"Evolution at Spike Position 519 in SARS-CoV-2 Facilitated Adaptation to Humans"***
Chelsea Cereghino, Department of Biomedical Sciences and Pathobiology, Virginia Tech, ID IGEP affiliated student

4:03 **"*Spatiotemporal Emergence of Lyme Disease in Appalachia (2000-2019)"***
Geoffrey Omondi Otieno, Department of Geography, Virginia Tech, ID IGEP affiliated student

4:15 – 4:30 **Break**

Closing Keynote

Latham AB

4:30 – 5:00 ***"Novel brain-penetrant antivirals for Venezuelan and eastern equine encephalitis"***
Colleen B. Jonsson, Van Vleet Chair of Excellence in Virology
University of Tennessee Health Sciences Center

5:00 – 5:15 **Closing Remarks**

5:15 – 7:00 **Social**
Latham CDEF

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Keynote Speakers

(in Latham AB)

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Morning Keynote

Latham AB

9:00 – 9:30

"Early warning systems for disease (re)emergence"

Pejman Rohani, Regents' Professor
University of Georgia

Pej Rohani is Regents' professor and the UGA athletics association professor at the university of Georgia. He is also the Associate Dean for Academic Affairs in the Odum School of Ecology and the Deputy Director of Center for Influenza Disease and Emergence Research. He works on the population biology of infectious disease systems, using computational modeling and statistics. His main research interests have focused on the transmission dynamics of microparasitic diseases, including influenza, pertussis and covid-19. He has co-authored a book and published more than 165 papers, including publications in Science, Nature, Lancet, PNAS and PLoS Biology. He has received fellowships from the Guggenheim Foundation, the American Association for the Advancement of Science and the Ecological Society of America.

Afternoon Keynote

Latham AB

1:20 – 1:50

"Airborne Disease Transmission from 6 Feet to 6 Million Feet"

Linsey Marr, Charles P. Lunsford Professor and University Distinguished Professor, Department of Civil & Environmental Engineering, Virginia Tech

Linsey Marr is a University Distinguished Professor and the Charles P. Lunsford Professor of Civil and Environmental Engineering at Virginia Tech. Her research group studies pollutants in indoor and outdoor air. Prior to the pandemic, she was one of a small number of researchers who studied viruses in the air. Marr is a member of the National Academy of Engineering and a Fellow of the American Association for Aerosol Research, American Geophysical Union, and International Society of Indoor Air Quality and Climate. She received a B.S. in engineering science from Harvard College and a Ph.D. in civil and environmental engineering from the University of California, Berkeley and completed her post-doctoral training at the Massachusetts Institute of Technology.

Closing Keynote

Latham AB

4:30 – 5:00

"Novel brain-penetrant antivirals for Venezuelan and eastern equine encephalitis"

Colleen B. Jonsson, Van Vleet Chair of Excellence in Virology
University of Tennessee Health Sciences Center

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Morning Session

Oral Presentations

(in Latham AB)

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IT'S A RISK TO HANDLE MONEY: NETWORKS OF INFORMATION AND INFECTION DURING THE 1889 INFLUENZA PANDEMIC

Ewing ET
Department of History, Virginia Tech, Blacksburg, VA

Background: In December 1889, as the so-called "Russian influenza" epidemic reached the United States, newspapers regularly published reports explaining and predicting the trajectory of this infectious disease. Scientific researchers, public health officials, and medical doctors provided guidance on how this respiratory disease spread, described common symptoms, and predicted the likely outcomes. Reputable and accurate guidance about disease transmission was accompanied by unreliable, speculative, and misinformed observations, rumors, and predictions. This paper examines reports that influenza spread among bank tellers who were handling bank notes as an example of networks of information and infection characteristic of a disease outbreak.

Methods: This project uses digitized historical newspapers to track reports about the possible spread of influenza by bank notes. These reports seemingly originated with a December 20, 1889 article in the Detroit Free Press referencing a large number of influenza cases among bank tellers. In the next few days, a wire service report with more details about the number of sick tellers in several banks were published in newspapers across the United States. These newspaper articles are analyzed in the context of late nineteenth century science about infectious diseases, which include advances in bacteriological understanding that identified microbes as the cause of disease transmission. In this era of rapidly changing scholarly inquiry, newspapers contributed to the distribution of scientific research, yet also disseminated inaccurate reports or unreliable speculation.

Results: Factors other than infected bank notes may explain the disease outbreak among Detroit bank tellers. Most banks in Detroit were located in the same downtown district and bank tellers had frequent interactions with customers, both of which likely contributed to the spread of disease.. Absences due to illness were easily observed among bank tellers; similar reports appeared for other occupations, such as police officers, school teachers, or postal clerks, whose absence in public spaces prompted reporting in newspapers. As the disease spread more widely throughout the population, these reports identifying a specific route of infection in a particular occupation gradually disappeared as it became obvious that everyone was susceptible to this disease. The reports on infected bank notes provide a substantive basis for understanding how networks of information related to the networks of infection during a pandemic.

Discussion: Although laboratory tests have revealed that the influenza virus may persist for several days on bank notes as well as other surfaces, the likelihood of becoming infected after touching cash is extremely low. Despite this level of scientific certainty, anxieties about contagion appeared again in the early stages of Covid-19, as many people stopped using currency for fear of catching the disease. Incomplete, inaccurate, and inconsistent guidance from public health organizations and commercial enterprises confirmed, reinforced, and exacerbated these reactions at a time of heightened concern, fear, and even panic. This presentation combines a historical case study with analysis of the human dimensions drawing upon experiences observed and lessons learned during the Covid-19 outbreak.

THE IMPACT OF HUMAN MOBILITY ON COVID-19 DYNAMICS

Saucedo, O.[1,2]

[1]*Department of Mathematics, Virginia Tech, Blacksburg, VA;* [2]*Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA*

Throughout our daily lives, we establish connections with other individuals either by visiting our favorite coffee shop, attending a local social gathering, or meeting with family and friends. We can view these interactions as a person's general social network. Certain events such as the global COVID-19 pandemic can disrupt these networks by either reducing the number of connections or disconnecting important connections. As observed in the recent pandemic, regions that enacted strong health measures with regard to minimizing social interactions helped reduce the transmission of the infectious disease. In this presentation, we will examine how perturbations to the human mobility structure influenced the spread of COVID-19.

Using Routinely Collected Public Health Data For Epidemiologic Research During The Evolving COVID-19 Pandemic: Challenges, Lessons, And Next Steps In Estimating Vaccine Effectiveness Using Surveillance Data From The Virginia Department Of Health

Silverman R.[1], Ceci A.[2], Helmick M.[3], Short E.[3], Finkielstein C.[2,4]

[1]*Center for Biostatistics and Health Data Science, Department of Statistics, Virginia Tech, Blacksburg, VA*
[2] *Fralin Biomedical Research Institute, Virginia Tech, Roanoke, VA*
[3] *Virginia Department of Health, Richmond, VA*
[4] *Department of Biological Sciences, Virginia Tech, Blacksburg, VA*

The evolving COVID-19 pandemic requires rapid and continuous data assessment to understand current conditions and inform effective risk-mitigation strategies. Given the propensity for COVID-19 to mutate, we must establish and maintain rapid data analytics systems and epidemiologic research across diverse settings to effectively sustain manageable conditions for endemic COVID-19. A major challenge for COVID-19 mitigation is decreasing vaccine effectiveness (VE) over time due to new variants and waning immunity. Transmission dynamics, population risk behaviors, policies, and resources all influence the impact of disease and mitigation tools like vaccines. Research is lacking from lower-resourced rural settings, despite distinctive transmission dynamics and public health resources compared to population centers. Virginia Tech's Molecular Diagnostics Lab within the Fralin Biomedical Research Institute (MDL-FBRI) has partnered with the Virginia Department of Health (VDH) since April 2020 to provide accurate SARS-CoV-2 molecular diagnostics and genomic sequencing. The accumulated surveillance, outbreak, clinical, diagnostic, and genomic data from normal operations at VDH and its partnership with MDL-FBRI remain underutilized for research that can benefit human health. While working with data collected for non-research purposes can be challenging, we aim to incorporate robust epidemiologic research methods to further support Southwest Virginia's public health infrastructure and inform evidence-based mitigation strategies locally and in other settings facing similar public health challenges. Our primary aims are to assess VE in a variety of risk-settings and demographic populations in the context of different dominating viral-variants. Applying lessons learned from our prior work estimating VE during a COVID-19 outbreak in a correctional facility in Southwest Virginia, our next study will estimate VE against reported COVID-19 infection among the pediatric population using VDH surveillance data.

COVID-19 vaccines continue to reduce the risk of infection and severe disease in all age-groups, to varying degrees depending on number of doses, time-since and type-of most-recent dose, and viral variant. However, the SARS-CoV-2 virus continues to mutate with variants increasing in transmissibility and in some cases, disease severity and VE has declined over time as individuals' immunity wanes and new variants with immune escaping capabilities have emerged. In response, the United States Food and Drug Administration (FDA) now recommends updated vaccines every fall, targeting recently dominating variants. While the vaccine's benefits are well-established, coverage among children is extremely low across the United States, with only 5.5% of those 6 month-4 years old completing a primary series and 0.6%, considered up-to date with the bivalent booster. Vaccine approval for children 6 months-4 years old lagged the general adult and older adolescent population by 14 months, several months after the 2021/22 winter Omicron variant surge, by which time an estimated 75% of children and adolescents in

the United States had already been infected at least once during an explosion in viral diversity. Studies on VE among pediatric populations are lacking and additional evidence can assist policy makers, healthcare providers, and the public in making informed decisions on pediatric COVID-19 vaccinations. Our observational epidemiologic studies aim to help fill these research gaps in the context of staggered vaccine eligibility and viral evolution.

3D Genome Architecture In Individual Development Of A Malaria Mosquito

Sharakhov I., Lukyanchikova V., Brusentsov I.
Department of Entomology, Virginia Tech, Blacksburg, VA

Abstract Text:
The 3D structure of chromatin observed in malaria mosquitoes has several characteristics of chromatin organization that have been previously reported in *Drosophila*. However, the *Anopheles* mosquito also reveals novel concepts of 3D genome organization, such as the existence of heterochromatic B-compartments, which are spatially separated from euchromatic B-compartments, as well as evolutionarily conserved and developmentally stable giant multimegabase-long chromatin loops (Lukyanchikova et al 2022). Here, we investigated aspects of the 3D genome architecture that are dynamic throughout mosquito individual development. We performed Hi-C on embryonic, larval, and adult stages of the mosquito development as well body parts of adult females and males including heads, antennas, proboscises and maxillary palps, thoraxes, and gonads of *Anopheles coluzzii*. Comparison of Hi-C maps obtained from adult and embryonic tissues confirmed that several autosomal and X-chromosomal giant loops are present across developmental stages of the mosquito. However, we also identified long-range chromatin interactions, particularly on the 3R arm, that occur at specific stages or in certain body parts during mosquito development. Some giant loops are soma-specific as they are absent in ovaries or testes but present in thoraxes and heads of adult mosquitoes. Moreover, heads have stronger contacts as well as additional giant loop that are absent in thoraxes suggesting their possible function in the nervous system or sensory perception. To explore this further, we compared Hi-C maps of antennas and heads without antennas. Antennas have receptors that are responsible for mosquito olfaction and play an important role in host-seeking, foraging, oviposition, and mating behaviors (Konopka et al 2021). We found that antennas and heads without antennas share only most but not all long-range looping interactions in the 3R chromosomal arm. Mosquito heads have additional giant loops that are absent in antennas. GO enrichment analysis has shown that anchors of giant head-specific loops are enriched in genes with roles in cell-cell signaling, sensory perception, neuron differentiation, signal transduction, and response to stimulus. The dynamic nature of these looping interactions in different cell types and during individual development suggests their functional significance for mosquito biology.

References:
Lukyanchikova V, Nuriddinov M, Belokopytova P, Taskina A, Liang J, Reijnders MJMF, Ruzzante L, Feron R, Waterhouse RM, Wu Y, Mao C, Tu Z, Sharakhov IV, Fishman V. *Anopheles* mosquitoes reveal new principles of 3D genome organization in insects. Nat Commun. 2022 Apr 12;13(1):1960. doi: 10.1038/s41467-022-29599-5.
Konopka JK, Task D, Afify A, Raji J, Deibel K, Maguire S, Lawrence R, Potter CJ. Olfaction in *Anopheles* mosquitoes. Chem Senses. 2021 Jan 1;46:bjab021. doi: 10.1093/chemse/bjab021.

Divergent Evolution of the Typhoid Toxin and Alternative Binding Subunit ArtB was Shaped by the Gain and Loss of Prophage Across *Salmonella* spp. Lineages

Cheng R.[1,2], Orsi R.[3], Hepp S.[4], Liao J.[2,4], and Wiedmann M.[3]
[1]Department of Food Science and Technology, Virginia Tech, Blacksburg, VA; [2]Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA; [3]Department of Food Science, Cornell University, Ithaca, NY; [4]Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA

ABSTRACT

Typhoid toxin is a novel A_2B_5 toxin that was first discovered and characterized in *Salmonella enterica* serovar Typhi, and was therefore predicted to play an integral role in typhoid fever. Subsequent genomic analyses have since established that the toxin is encoded by >100 nontyphoidal serovars, suggesting that it may play a broader role in *Salmonella* pathogenicity. We examined the distribution, conservation, and selective pressure of typhoid toxin subunits throughout *Salmonella* spp. isolates. We found that typhoid toxin genes are broadly distributed throughout serovars in lineages within species *bongori* and *enterica*, with the majority mapping to genomic regions annotated as incomplete prophage suggesting that phage likely played an important role in the acquisition of this toxin early in *Salmonella*'s evolutionary history. We also found that 94% of isolates that encoded typhoid toxin genes also encoded a second binding subunit *artB*, which was acquired separately and has been selectively maintained by the majority of typhoid-toxin positive isolates. Conservation of amino acid residues associated with catalytic activity and glycan binding in all typhoid toxin subunits suggest that this toxin is likely functional in most isolates. Finally, although select variants of PltB and ArtB show evidence of directional selection, branch-specific models do not support the hypothesis that PltB from *S.* Typhi evolved to selectively bind human cells; rather this adaptation likely occurred earlier in typhoid toxin's evolutionary history. Overall, the conservation of typhoid toxin in *Salmonella* spp. suggests that this toxin likely plays a broader role in *Salmonella* biology than previously thought.

IMPORTANCE

The role of typhoid toxin in human disease remains unclear given the broad distribution of this toxin in nontyphoidal subsp. *enterica* serovars that do not cause typhoid fever. Our study adds further support that this toxin likely plays a role in host-pathogen interactions beyond those involved in the development of typhoid fever, given the conservation of typhoid toxin genes throughout multiple species and subspecies of *Salmonella*. Furthermore, our data support that acquisition of typhoid toxin and ArtAB likely occurred early in *Salmonella*'s evolutionary history, most likely in the ancestor of *S. bongori* prior to the divergence of *S. bongori* and *S. enterica*. Finally, the high conservation of *pltB* throughout subspecies *enterica* serovars as well as branch-specific substitution models suggest that evolutionary events that led to PltB's ability to bind glycans on human cells occurred prior to the emergence of serovar Typhi.

COVID-19: AGENT-BASED SIMULATION-OPTIMIZATION TO VACCINE CENTER LOCATION VACCINE ALLOCATION PROBLEM

Yin X.[1], Bushaj S.[2], Yuan Y.[3], and **Büyüktahtakın Toy E.[4]**

[1]Management Science and Information Systems, Oklahoma State University, Norman, OK, USA; [2]School of Business and Economics, SUNY Plattsburgh, Plattsburgh, New York, USA; [3]Altfest Personal Wealth Management, NY, USA; [4]Grado Department of Industrial and Systems Engineering, Virginia Tech, Blacksburg, VA, USA

Abstract

In this talk, we will present an agent-based simulation-optimization modeling and algorithmic framework to determine the optimal vaccine center location and vaccine allocation strategies under budget constraints during an epidemic outbreak. Both simulation and optimization models incorporate population health dynamics, such as susceptible (S), vaccinated (V), infected (I), and recovered (R), while their integrated utilization focuses on the COVID-19 vaccine allocation challenges. We first formulate a dynamic location-allocation mixed-integer programming (MIP) model, which determines the optimal vaccination center locations and vaccines allocated to vaccination centers, pharmacies, and health centers in a multi-period setting in each region over a geographical location. We then integrate it with the agent-based epidemiological simulation model of COVID-19 (Covasim). Covasim involves complex disease transmission contact networks, including households, schools, and workplaces, and demographics, such as age-based disease transmission parameters. We combine the extended Covasim with the vaccination center location-allocation MIP model into one single simulation-optimization framework, which works iteratively forward and backward in time to determine the optimal vaccine allocation under varying disease dynamics. The agent-based simulation captures the inherent uncertainty in disease progression and forecasts the refined number of susceptible individuals and infections for the current time period to be used as an input into the optimization. We calibrate, validate, and test our simulation-optimization vaccine allocation model using the COVID-19 data and vaccine distribution case study in New Jersey. The resulting insights support ongoing mass vaccination efforts to mitigate the impact of the pandemic on public health, while the simulation-optimization algorithmic framework could be generalized for other epidemics.

CZAP Center for Emerging, Zoonotic, and Arthropod-borne Pathogens

Poster Presentations

(by last name)

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Number	Name	Title
1	Abdelsattar, Abdallah	Repurposing Valnemulin for Combating Multidrug-Resistant *Neisseria gonorrhoeae.*
2	Abouelkhair, Ahmed*	Antifungal Outweighs the Drawbacks of Vancomycin as a Promising Therapeutic Option for Combating *Clostridioides Difficile* Recurrence
3	Alkashef, Nour*	Repurposing Pimvanserine to Enhance Fluconazole Activity Against *Cryptococcu*s
4	Ataei Kachouei, Matin*	MXene Enabled 3D Biosensors for Rapid and Early Detection of Subclinical *Mastitis* in Dairy Cows
5	Barker, Colby	Using Synthesis to Map the Human Sphingolipidome
6	Bataglioli, Rogerio	Temperate Phage Engineering for Modulating Undesired Genes in the Commensal Bacteria
7	Belay, Kassaye	A whole genome-based taxonomy of the genus *Fusarium*
8	Bianculli, Rachel*	Structure-property Relationship of Sialic Acid Containing Polymers for Influenza Inhibition
9	Bilyeu, Landon	Monitoring Wind And Particle Counts Near Freshwater And Marine Harmful Algal Blooms (HABs)
10	Breiner, Logan *	Lead Compound Discovery from Pleuromutilin by Utilizing "Click Chemistry"
11	Brennan, Reilly*	Estimating Pathogen-Spillover Risk Using Host-Ectoparasite Interactions
12	Campbell, Rose	Impacts of Bacterial Metabolites on the Coral Microbiome as Clues to Understanding Coral Diseases
13	Carney, Shannon *	Development and Characterization of a Sublethal-Sequelae Mouse Model of EEEV Infection

14	Carpenter, Carrie	Investigating *Pseudomonas syringae's* Ability to Form Biofilms on Microplastics
15	Channell, Kirsta and Sandusky, Lindsay	Using Reverse Metabolomics to Discover New Small Molecules from the Gut Microbiome
16	Chick, Shannon	Highly Sensitive and Low-Cost Nanomaterials-Enabled Biosensors for Detection of *Subclinical Ketosis* in Dairy Cows
17	Diggs, Shajaesza	Effects of Fluid Properties on Mosquito Feeding: Modulation of the Cibariarl and Pharngeal Pumps' Dynamics
18	Elgammal, Yehia*	Saquinavir Potentiates Itraconazole's Antifungal Activity Against Multidrug -Resistant *Candida Auris In Vitro* and *In Vivo*
19	Farthing, Wilson*	Discovering the Role of Leucine-Responsive Regulatory Protein (Lrp) During Corn Xylem Infection
20	Faw, Lindsey*	Transmission of La Crosse Virus in *Ae. triseriatus*, the Native Vector, and *Ae. albopictus*, an Invasive Candidate Vector.
21	Fike, Katie	Unpeeling the Layers of Lysophospholipid Metabolism in *Plasmodium falciparum*-Infected Erythrocytes
22	Flor, Rafaela*	Rift Valley Fever Virus L Polymerase Phosphorylation Regulates Viral RNA Production in a Protein Phosphatase-1 Manner
23	Franklin, Hollyn*	Model to Study Impact Of Gut Phages On Host Health
24	Gangwar, Priyanka	Multilocus Sequence Analysis Reveals Six *Fusarium* Species Complexes Associated with the Outbreak of Fusarium Wilt in Flue-Cured Tobacco
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Repurposing Valnemulin for Combating Multidrug-Resistant *Neisseria gonorrhoeae*.

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Abstract

The Centers for Disease Control and Prevention (CDC) has categorized *Neisseria gonorrhoeae* as an urgent public health threat due to increasing incidence of infections and the uprising bacterial resistance to antibiotics. With approximately 82.5 million annual infections, *N. gonorrhoeae* has developed resistance to all classes of antibiotics. Currently, ceftriaxone is the only recommended treatment, but the emergence of high-level resistance poses a serious challenge. Without developing new anti-gonorrheal treatments, the world faces the real possibility of an untreatable gonococcal infection. Drug repurposing represents an effective approach to drug discovery as it reduces the time, costs, and risks associated with traditional drug innovation. Adopting the drug repurposing approach, we identified the animal-approved drug valnemulin as a potent anti-gonococcal agent. Valnemulin is an FDA-approved pleuromutilin derivate used to treat swine dysentery, colitis and pneumonia. It displayed a potent activity inhibiting a panel of 24 multidrug-resistant clinical isolates of *N. gonorrhoeae* with concentrations ranging from 0.03 to 0.5 μg/mL. The drug exhibited a rapid bactericidal activity against *N. gonorrhoeae,* completely eradicating the high bacterial burden within 4 hours and demonstrated a prolonged post-antibiotic effect (> 6 hours). Importantly, no resistant mutants emerged even in the presence of a high bacterial inoculum. Valnemulin's advantages extend beyond its efficacy. In contrast to ceftriaxone, it demonstrated limited activity against the protective commensal vaginal microbiota, a crucial defense against gonococcal infection. Additionally, valnemulin was superior to ceftriaxone in killing the intracellular *N. gonorrhoeae,* completely clearing infected endocervical cells within 5 hours. Furthermore, valnemulin reduced the expression of IL-6, a pro-inflammatory cytokine contributing to the severity of gonococcal infection. Collectively, our findings indicate that valnemulin represents a promising anti-gonococcal agent that merits further investigation.

Antifungal Outweighs The Drawbacks Of Vancomycin As A Promising Therapeutic Option For Combating *Clostridioides Difficile* Recurrence

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Abstract

Clostridioides difficile is considered as an urgent public health threat and it is a leading cause of healthcare-associated infection worldwide. *C. difficile* has been linked to over 500,000 infection cases and 29,000 cases of fatalities globally. Vancomycin and fidaxomicin, are the only FDA-approved antibiotics for the treatment of *C. difficile* infection (CDI). However, the treatment of CDI is compromised by the high treatment failure and recurrence associated with vancomycin and fidaxomicin. Moreover, the emergence of resistant strains further complicates the landscape... In light of these challenges, novel therapeutics for treatment of CDI are desperately needed. To address this need, we screened a library of FDA-approved drugs and identified abafungin, an antifungal agent, as a potent inhibitor for *C. difficile*. Abafungin exhibited a potent anticlostridial activity against a wide panel of pathogenic *C. difficile* strains, inhibiting the growth of 90% of these strains (MIC_{90}) at the concentration of 0.25 μg/mL. Notably, in contrast to the drugs of choice for treatment of CDI, abafungin did not affect the growth of the beneficial bacteria that compose the host intestinal microbiota. Intriguingly, abafungin, at subinhibitory concentrations, surpassed vancomycin and fidaxomicin in inhibiting the *C. difficile* toxins formation (by 50 %), and spores' production (by 5 Log reduction), both of which are the two main virulence components driving CDI and its recurrence. Furthermore, investigating the physicochemical properties of abafungin revealed that pre-exposure to simulated gastric intestinal fluid and simulated intestinal fluid did not affect the potency of abafungin against *C. difficile.* Moreover, abafungin interacted synergistically with vancomycin and metronidazole against *C. difficile in vitro.* Concluding our study, we investigated the *in vivo* efficacy of abafungin in a *C. difficile* recurrence model. Remarkably, abafungin significantly protected mice against *C. difficile* recurrence resulting in 83.3% survival rate. In contrast, the drug of choice, vancomycin resulted in 33.3% survival rate. Collectively, our findings indicate that abafungin represents a promising anti-CDI therapeutic that warrants further investigation.

REPURPOSING PIMVANSERINE TO ENHANCE FLUCONAZOLE ACTIVITY AGAINST CRYPTOCOCCUS.

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Cryptococcal disease poses a significant threat to immunocompromised individuals. *Cryptococcus neoformans* (*C. neoformans*) is a key culprit in this illness, primarily affecting the lungs as well as the central nervous system, causing life-threatening meningitis. Nearly 150,000 new cases of cryptococcal meningitis (CM) are reported every year around the world, emphasizing its substantial impact. The current standard treatment approach includes the administration of amphotericin B alongside other antifungals, mainly flucytosine, to expedite fungal clearance and mitigate potential toxicity. Although fluconazole is recommended as an alternative, its fungistatic nature often results in treatment failure and relapse.

In the present work, we identified pimvanserine (PVT), an atypical antipsychotic, as an adjuvant to enhance fluconazole monotherapy. Here, we found that PVT synergistically interacts in conjugation with fluconazole against 85% of the investigated strains of *C. neoformans/gattii* complex as evaluated by checkerboard assay. Moreover, PVT, both alone and in combination with fluconazole, significantly impacts the metabolic activity of mature *Cryptococcus* biofilm. Our investigation also demonstrated that the PVT/fluconazole combination effectively reduces the fungal burden in *Caenorhabditis elegans* infected with C. *neoformans.* Delving into the mechanism of PVT's synergy with fluconazole, our study unveiled that PVT disrupts the mitochondrial membrane potential and induces the accumulation of reactive oxygen species (ROS) in a dose-dependent manner. Additionally, PVT influences the integrity of the cell membrane, contributing to the loss of cell viability. This work highlights the significance of PVT, with its ability to cross the blood-brain barrier, as a potential adjuvant to improve fluconazole monotherapy. The synergy exhibited by PVT and fluconazole offers a potential avenue to address the challenges posed by Cryptococcus neoformans/gattii complex infections.

MXene Enabled 3D Biosensors for Rapid and Early Detection of Subclinical Mastitis in Dairy Cows

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Mastitis is an inflammation of the mammary gland caused by a number of pathogens including *Streptococcus*, *Staphylococci*, and coliforms. Sub-clinical *mastitis* (SCM) is the most economically challenging disease in modern dairy herds and can pose a risk to other dairy cows as it is difficult to detect due to its asymptomatic nature, causing extreme difficulty in prevention and control, and long-term reductions in milk yield. The cost of SCM in the US alone exceeds $2 billion annually. Somatic cell count, California Mastitis Test, and Wisconsin Mastitis Test are the diagnostic tests for sub-clinical mastitis. Notably, most of these tests are periodic tests rather than continuous or frequent measurements of sub-clinical mastitis in dairy herds. These tests provide qualitative measurements, and they have their own sensitivities (less than 90%) and specificities (85%). Thus, accurate, early, continuous, and on-site detection of sub-clinical mastitis can increase milk yield, limit the spread of infection, facilitate herd and health management, and improve the well-being of dairy cows.

We have developed a *miniature,* farm-deployable 3D printed sensor for on-site testing of subclinical *mastitis* in dairy cows. This sensor has two detectors and is made of MXene (*i. e.* 2D transition metal carbides or Ti_3C_2) sheets. This sensor measures elevated N-acetyl-β-D-glucosaminidase (*NAGase*) in milk and pH which are early indicator biomarkers of *mastitis*. *NAGase* is secreted when somatic cells (immune cells) increase in milk due to pathogenic infection. The analytical sensitivity of the sensor is 0.01 μg/mL and the response time is less than 1 min. The details of the sensing results of the device will be discussed at the conference. This innovative device is to reduce the time and effort required to detect *mastitis* in dairy cows at milking to control the spread of pathogen infections.

Keywords: Mastitis, Pathogens, 3D sensors, Dairy Cows

Using Synthesis to Map the Human Sphingolipidome

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Once thought to be present only in the brain, sphingolipids are now known to be found throughout eukaryotic and some prokaryotic cells with diverse structures and functions. It is predicted that sphingolipids can have tens of thousands of distinct structures, many of which remain unknown. To identify these previously unknown molecules, sphingolipids can be made using chemical synthesis then an MS/MS spectrum of the authentic compound can be compared with publicly available metabolomics data to identify if a structure or analogous structure has been found in human samples. A new strategy for synthetically accessing the key sphingoid base structure is presented here. In this divergent method, photoredox-catalyzed decarboxylation of amino acids is followed by radical addition to the C-O bond of aldehydes to create the sphingolipid core in a single synthetic step. With this route, a diverse library of sphingolipid structures will be prepared and searched for in public metabolomics data. This technique provides the location of sphingolipids in the body as well as any disease associations. Taken together, this work allows for a faster and easier synthesis of sphingolipids to enable the study of complex sphingolipid pathways in humans and microbiota-mediated diseases.

Temperate Phage Engineering for Modulating Undesired Genes in the Commensal Bacteria

Commensal bacteria play an essential role in human health, contributing to the absorption of nutrients, immunological functions, and the clearance of pathogens. Key to the continued symbiotic relationship between the mammalian host and their microbes is the preservation of microbial diversity and its resilience against environmental perturbations that may result in dysbiosis-related diseases. Contrary to the diseases caused by pathogens that are not normal members of the healthy human gut, commensal bacteria may be broadly beneficial but also linked to chronic diseases. Often, it can be specific bacterial genes in these commensal bacteria that are a factor in these diseases. Delivering genetic material directly to bacteria is an emerging strategy for modulating the expression of specific bacterial genes deleterious to the mammalian host. Phages, the viruses of bacteria, are a potential strategy for precisely targeting bacterial function in their native environment with minimal disruption to the mammalian host and the microbiota. Naturally present in the gut, temperate phages use the lysogenic cycle to remain dormant in the bacterial genome and disseminate new genetic functions in the targeted bacteria population. This work aims to use phages to deliver heterologous genes to commensal bacteria to modulate the expression of genes linked to the production of genotoxic compounds. To accomplish this, we first used a combinatorial approach to investigate phages that can infect colibactin-producing strains, a DNA-alkylator compound associated with the occurrence of colorectal cancer. By identifying a hybrid lambdoid phage that infects the commensal *E. coli* NC101, this temperate phage was host-adapted to increase the levels of phage infection and lysogeny efficiency in the targeted host. We are currently working on engineering this temperate phage to modulate the expression of a reporter gene in the targeted commensal bacteria for later testing the modulation of the gene associated with colibactin production. These findings will elucidate the opportunities and challenges for temperate phage use to control gene expression on commensal bacteria for later testing alternatives to ameliorate chronic diseases by targeting gene expression in the causative microbial in the mammalian gut.

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A whole genome-based taxonomy of the genus *Fusarium*

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The genus *Fusarium* encompasses fungal pathogens that are critically significant due to their potential for plant affliction and mycotoxin production, adversely impacting both animal and human health. Despite substantial research advancements regarding Fusarium, taxonomic clarity concerning its species and related complexes remains elusive and contentious. However, genome sequencing and analysis might alleviate the challenges posed by conventional taxonomic techniques. An ideal genome-based taxonomy should be swift, exact, and reliable, ensuring prompt identification and containment of potential disease outbreaks before reaching alarming levels. Such identification can be achieved either via (1) whole genome sequencing following the isolation of pure cultures or (2) through metagenomic sequencing, eliminating the need for direct culturing of the pathogen. In our study, we meticulously established a genome-scale phylogeny for the Fusarium genus, delving into ambiguously resolved interspecies relationships. Using BUSCO (Benchmarking Universal Single-Copy Orthologs) for single-copy gene detection, MUSCLE for BUSCO gene alignment, TrimAI for eliminating misaligned sequences, and IQ-Tree, we crafted a core-genome tree encompassing 276 fungal species and subspecies. Additionally, we employed the computational prowess of sourmash, a k-mer based centric methodology, coupled with the Life Identification Number (LIN) system for nuanced genome-based classification. Our findings indicate a commendable concordance between sourmash and LINflow outputs, mirroring the core-genome tree built upon 391 single copy genes. Through our research, we aim to demonstrate the viability of the proposed methodologies in enhancing the genome-based classification of Fusarium and other fungal genera, a step pivotal for bolstering plant health, ensuring food safety, and preserving food security.

STRUCTURE-PROPERTY RELATIONSHIPS OF SIALIC ACID CONTAINING POLYMERS FOR INFLUENZA INHIBITION

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Influenza replication is initiated by the polyvalent interactions between hemagglutinin on the viral surface and sialic acid on the epithelial cell surface of the lungs. This polyvalent interaction can be mimicked synthetically using polymers, where each repeat unit acts as a place for potential binding. By appending sialic acid to polymers, we can create "decoy" receptors that will bind to the surface of influenza via hemagglutinin and reduce the available viral surface that can interact with the cell surface. Linear sialic acid-containing polymers have been extensively studied for influenza inhibition, but due to varied structural motifs and nonuniform experimental assay conditions, it is difficult to conclude how polymer parameters affect overall antiviral properties.[1] By synthesizing materials with controlled and well characterized polymer properties (molecular weight, sialic acid content, comonomer identity, linker length), we can better elucidate how these parameters impact the mechanism of inhibition and efficacy of the material. To do this, we have synthesized a library of sialic acid-styrene sulfonate copolymers with systematically varied parameters and measured their influenza inhibition using a variety of assays (hemagglutination inhibition, plague, etc.). This data help elucidate structure-property relationships and help optimize sialic acid-containing polymers for enhanced influenza inhibition.

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Monitoring Wind And Particle Counts Near Freshwater And Marine Harmful Algal Blooms (HABs)

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Abstract

Harmful algal blooms (HABs), caused mostly by toxic cyanobacteria, are a threat to aquatic ecosystems. Toxins from HABs may be aerosolized and transported downwind. New information is needed about the environmental conditions associated with the aerosolization and transport of HAB cells and toxins. We used a ground-based sensor package to monitor weather, measure airborne particles, and collect air samples on the shore of a freshwater HAB (bloom of *Rhaphidiopsis*, Lake Anna, Virginia) and a marine HAB (bloom of *Karenia brevis*, Gulf Coast, Florida). The sensor packages contained a sonic anemometer, an impinging device, and an optical particle counter. A drone was used to measure windspeed and wind direction at different altitudes above the freshwater HAB. Drone measurements of the wind column showed differences in wind speed and direction at different altitudes, highlighting the need for wind measurements at multiple heights to capture the full extent of weather phenomena. At the Florida sites, airborne particle counts increased throughout the day and the wind direction (offshore versus onshore) was strongly associated with these counts with offshore wind sources having 3-4 times the number of aerosolized particles compared to onshore wind sources at one beach sampling location. A predictive model, trained on a random set of matched weather and particle counts, was able to predict airborne particle counts with an R Square of 0.581 for the freshwater HAB in Virginia and an R Square of 0.802 for the marine HAB in Florida. Additional work is needed to better understand the long-term fate and transport of aerosolized cyanobacterial cells and toxins and how weather influences said transport.

Lead Compound Discovery from Pleuromutilin by Utilizing "Click Chemistry"

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Pleuromutilin is a potent natural product antibiotic derived from the mushroom *Clitopilus scyphoides* and is a member of the diterpenoid class. Semisynthetic drugs based around the pleuromutilin core have been approved as human and veterinary antibiotics. Their unique mechanism of action has a low prevalence of resistance due to a slow development of resistance and a lack of use as livestock growth enhancers. This quality makes them a promising target for continued drug development. Previously, our work on pleuromutilin-functionalized triazoles demonstrated that C22 substituted compounds maintained activity while C20 substitution abolished it. By using computation and structure-based drug design to build on these findings, we show that epimerization of the C12 position provides potent lead compounds with C20 triazoles. These provide a new class of pleuromutilins to investigate, the 12-*epi*-20-triazolyls. Additionally, electrophilic intermediate compounds demonstrated enhanced antibiotic activity. Based on these results, a series of electrophilic pleuromutilin analogs were generated and are undergoing testing. We hypothesize that these derivatives will reveal ribosome binding details and possibly a new mechanism of pharmacophore action as the source of increased activity.

Estimating Pathogen-Spillover Risk Using Host-Ectoparasite Interactions

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Pathogen spillover corresponds to transmission of a pathogen or parasite from an original host species to a novel host species and preludes zoonotic disease emergence. Understanding the interacting factors that lead to pathogen transmission in a zoonotic cycle could help identify novel hosts of pathogens and the patterns that lead to disease emergence in humans. We hypothesize that ecological and biogeographic factors drive host encounters, infection susceptibility, and cross-species spillover transmission. Using a rodent-ectoparasite system in the Neotropics, with shared ectoparasite associations as a proxy for ecological interaction between rodent species, we assessed relationships between rodent species using geographic range, phylogenetic relatedness, and ectoparasite associations to determine the roles of generalist and specialist hosts in the transmission cycle of hantavirus. A total of 50 rodent species were ranked on their centrality in a network model based on ectoparasites sharing (i.e., 91 flea, 18 mite, 17 lice, and 5 tick species). Geographic proximity and phylogenetic relatedness were predictors for rodents to share ectoparasite species and were associated with shorter network path distance between rodents through shared ectoparasites. Independent data of seven known hantavirus hosts were successfully predicted using the rodent-ectoparasite network model. Five novel rodent species were predicted by the model as potential, unrecognized, hantavirus hosts in South America. Findings suggest that ectoparasite data, geographic range, and phylogenetic relatedness of wildlife species could help predict novel hosts susceptible to infection and possible transmission of zoonotic pathogens. Predictions of new rodent hosts can guide active epidemiological surveillance in specific areas and wildlife species to mitigate hantavirus spillover transmission risk from rodents to humans. This study supports the idea that ectoparasite relationships among rodents are a proxy of host species interactions and can inform transmission cycles of diverse pathogens circulating in wildlife disease systems, including wildlife viruses with epidemic potential such as hantavirus.

IMPACTS OF BACTERIAL METABOLITES ON THE CORAL MICROBIOME AS CLUES TO UNDERSTANDING CORAL DISEASE

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Coral reefs are a critical ecosystem to the health and biodiversity of the Earth. Unfortunately, numerous coral diseases have ravaged reefs in recent years, wiping out over 80% of coral cover in the Caribbean in just 40 years. Most diseases have no clear single pathogen and are believed to be caused by polymicrobial coinfections that disrupt the microbiome's balance. Several diseases have been shown to directly harm the endosymbiotic dinoflagellate, which produces up to 90% of the coral's nutrients, and this likely is a key step in disease progression. However, much is yet to be understood about the progression of these diseases and the key metabolites involved in the signaling that ultimately leads to the death of the coral. Towards this end, we have been investigating the chemical interactions between known coral pathogens, bacterial symbionts, and the endosymbiotic dinoflagellates to understand the exchanges occurring that lead to dysbiosis. Recently, we observed antimicrobial activity exhibited by *Vibrio coralliilyticus*, a known coral pathogen, against multiple bacterial symbionts. Further chemical investigation revealed a suite of amphibactins, a class of marine siderophores, that are likely responsible for this growth inhibitory activity. Current work is focused on understanding the ecological role of these siderophores and their impact on overall coral health.

Development and Characterization of a Sublethal-Sequelae Mouse Model of EEEV Infection

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The mosquito-borne encephalitic alphaviruses (eastern, Venezuelan, and western equine encephalitis viruses – EEEV, VEEV and WEEV) cause disease in both equines and humans resulting in overt encephalitis in a significant percentage of cases. EEEV infection results in death in 50-75% of patients and of the survivors, 50-90% experience debilitating neurological sequelae. These include seizures, paralysis, intellectual disability, and permanent mood and behavioral changes. The significant mortality and morbidity associated with EEEV infection underscores the need for useful interventions and currently, there are no therapeutic options available. Few studies have described EEEV pathogenesis in lethal mouse models and there is no neurological sequelae model currently available. Because a drug and vaccine candidate will need to be approved via the Animal Rule, well-characterized models that are reflective of human disease for EEEV are critical to the development of prospective therapeutics and vaccines. We aim to address this deficiency by developing and characterizing a novel neurological sequelae model of EEEV infection. We hypothesize that animals that appear to have recovered from EEEV infection still suffer substantial cognitive deficits resulting from neurodegeneration associated with viral infection. A pilot study was designed to measure neurological sequalae through behavioral alterations following EEEV infection, where 10-week-old CD-1 mice underwent cognitive testing before and after infection. Cognitive testing consisted of a modified SHIRPA test, elevated plus maze, and y-maze. Group 1 consisted of uninfected control mice, group 2 were subcutaneously infected with EEEV TaV-nLuc and imaged with an IVIS Lumina III, and group 3 were subcutaneously infected with EEEV TaV-nLuc and were followed for survival and clinical symptoms. Group 2 was imaged on days 2, 4, 6, and 8 post-infection (pi) with peak viral replication occurring at D6pi. Neurological symptoms were observed primarily at days 5-7pi and included bilateral rear limb paralysis, circling, head tilt, hyperexcitability, and possible seizure activity. All mice with detectible virus in the brain succumbed to infection. Cognitive testing from the mazes found that the uninfected mice and infected mice performed similarly post-infection, suggesting that anxiety and working memory were not affected post-EEEV infection in surviving mice. Because surviving mice showed little to no viral replication in the brain as determined through IVIS imaging, mice in future studies will be challenged via intranasal route of infection as a more direct route of infection to the brain. We will also test attenuated mutants in causing disease and long-term neurological impacts. Y-maze testing will be replaced with novel object recognition testing in our cognitive assessments to determine the impact of EEEV on memory. Developing a model of this nature is important because survivors of EEEV infection suffer from substantial cognitive deficits. Thus, characterizing the host response to EEEV infection is critical for understanding consequential neuropathology associated with infection.

Investigating *Pseudomonas syringae's* Ability to Form Biofilms on Microplastics

Microplastics are tiny plastic fragments that are common contaminants in natural and managed ecosystems. Some of these microplastics are associated with microorganisms, which may assist in the transport and/or degradation of the microplastics. The bacterium *Pseudomonas syringae* has been found in clouds, rain, and snow, and some strains are ice nucleation active—they express a protein that causes water to freeze at warmer temperatures. This study aimed to assess *P. syringae's* ability to attach and form biofilms on different plastic polymers (2x3 mm +/- 1mm). The strains used were captured from the environment and include a TLP2 wildtype (ice+, ice nucleation active) and a mutant (ice-, no ice nucleation). Studies were conducted to investigate the attachment of these strains of *P. syringae* to plastic polymers, followed by quantification of biofilm formation on the different plastics. Various methods were used to visualize the biofilm including light microscopy, Raman spectroscopy, and scanning electron microscopy. A standard 0.1% crystal violet assay was used to estimate the biomass accumulated on plastic by using UV spectroscopy to measure optical density changes at 600nm. Initial experiments demonstrated that *P. syringae* was able to attach to polypropylene, low-density polyethylene, high-density polyethylene, polystyrene, and polyethylene terephthalate. Biofilm production was found to be most prominent on high-density polyethylene followed by low-density polyethylene and polypropylene. *P. syringae's* ability to attach and form biofilms on these different polymers suggests that microplastics can act as a vector to transport the bacterium to new environments. Future studies aim to assess the capability of *P. syringae* to attach to micron-sized microplastics, investigate their viability after being aerosolized via wave breaks, and study the impact of microplastics on *P. syringae's* ability to catalyze ice nucleation.

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Elucidating Metabolites of the Gut-Brain Axis Utilizing Synthesis-Based Metabolomics

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Human enteric and central nervous systems are linked physically and chemically through the 'gut-brain axis.' Evidence suggests that crosstalk between these two systems can influence behavior, inflammation, and certain neurological disorders. Signaling along the gut-brain axis is thought to be influenced by microbiota derived small-molecule metabolites, many of which are undiscovered and uncharacterized. Here, reverse metabolomics is used to help identify these unknowns. Mixtures of pertinent small molecules (e.g. carboxylic acids, fatty acids, bile acids) found in the human gut were conjugated with biogenic amine neurotransmitters to synthesize potential metabolites present in the gut-brain axis. These synthetic mixtures were then characterized using Liquid-Chromatography Mass-Spectrometry (LC-MS) and MS/MS spectra were compared with public untargeted metabolomics data to find matches of synthesized compounds and uncover possible phenotypic associations. This high through-put method for annotating previously unknown metabolites will expand our knowledge of the human metabolome and provide insight into gut-brain signaling pathways.

Highly Sensitive and Low-Cost Nanomaterials-Enabled Biosensors for Detection of *Subclinical Ketosis* in Dairy Cows

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Ketosis or *acetonemia* is a metabolic disease that typically occurs shortly after calving. During the transition period of dairy cows, the energy demand for milk production increases significantly and cows enter a negative energy balance due to not consuming enough energy, which can cause excessive amounts of ketone bodies in a cow's body fluids. Severe ketosis can easily occur as cows will lose a large amount of body condition within a short period of time. This loss of body condition can lead to the cow taking longer to become pregnant, having lower milk quality, and can leave the cow susceptible to other infections such as mastitis. *Beta-hydroxybutyrate* is a potential biomarker to detect *subclinical ketosis* in blood samples. Thus, the quantification of subclinical ketosis can improve the health and productivity of dairy cows. In this study, an electrochemical biosensor is developed to detect *beta-hydroxybutyrate* in the serum samples of dairy cows. The design for this biosensor will include a microfluidic device with 2D-graphene flakes on the working electrode that will increase sensitivity. The biosensor will use a bi-enzyme solution made of one-part hydroxybutyrate dehydrogenase and one-part NADH. The goal of this work is to use the covalent immobilization of enzymes with 2D-graphene flakes to create a more stable, robust, and sensitive biosensor for *subclinical ketosis* in dairy cattle. We aim to develop biosensors to improve dairy herd management by detecting different animal diseases, such as mastitis or lameness, and antibiotic residue in animal excrement. In addition, farmers will be able to detect ketosis at its earliest stages with this sensor which can improve herd health management decisions. At the CeZAP Infectious Disease Symposium poster session, our interest is to present the sensor design, and potentially implement new biosensor devices into precision livestock farming.

EFFECTS OF FLUID PROPERTIES ON MOSQUITO FEEDING: MODULATION OF THE CIBARIARL AND PHARNGEAL PUMPS' DYNAMICS.

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To obtain the carbohydrates required to sustain their metabolism, mosquitoes imbibe various fluids, from plant nectar to honeydew, and tree sap. In addition, females of some species require blood meals from vertebrate hosts for egg production. Mosquitoes thus feed on fluids with a wide range of physical properties, including granulometry, temperature, and viscosity. Despite the epidemiological importance of their feeding behavior, there has been very little investigation into how mosquitoes are able to feed on such a broad range of fluids. In particular, whether biomechanical constraints are preventing nectar-feeding mosquito species from transitioning to blood-feeding remains to be determined. In this context, we hypothesized that the activity and properties of the feeding pumps constrain the types of fluids a mosquito can ingest. To test our hypothesis, we fed mosquitoes on sugar solutions varying in viscosity while conducting electromyography (EMG) recordings of the cibarial and pharyngeal (i.e., feeding) pumps. We compared males and females of two mosquito species: *Aedes aegypti*, which feeds preferentially on human hosts, and *Culex quinquefasciatus*, which feeds primarily on birds. Our findings indicate that each species has evolved different strategies to cope with variations in fluid properties. Results will be discussed from a biological and ecological standpoint, as it may have important implications for the development of new strategies for controlling mosquito-borne diseases by targeting the feeding behavior of mosquitoes.

SAQUINAVIR POTENTIATES ITRACONAZOLE'S ANTIFUNGAL ACTIVITY AGAINST MULTIDRUG-RESISTANT *CANDIDA AURIS IN VITRO* AND *IN VIVO*

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Abstract
Candida species are highly opportunistic yeasts that are responsible for serious invasive fungal infections among immunocompromised patients worldwide. Due to the increase of drug resistance and incidence of infections, there is an urgent need to develop new antifungals and to identify co-drugs that can sensitize drug-resistant *Candida* to antifungals. The objective of this study was to assess the effect of saquinavir on the activity of azole antifungals against *Candida auris.* The *in vitro* interaction of saquinavir and three azole antifungals (itraconazole, voriconazole and fluconazole) was evaluated against a panel of *C. auris* isolates. The itraconazole/saquinavir combination exhibited a synergistic relationship against all *C. auris* isolates tested with the fractional inhibitory concentration index (FICI) ranging from 0.03 to 0.27. Moreover, a time-kill kinetics assay revealed that saquinavir restored the itraconazole's fungistatic activity against *C. auris*. Furthermore, saquinavir restored itraconazole's antifungal activity against other clinically important *Candida* species. The mechanistic investigation indicated that saquinavir significantly inhibited efflux pumps, glucose utilization and ATP synthesis in *Candida*. Finally, a murine model of *C. auris* infection was used to evaluate the efficacy of the itraconazole/saquinavir combination in the presence of ritonavir (as a pharmacokinetic enhancer). The combination significantly reduced the fungal burden in the kidneys by 0.93-$\log_{10}$ colony forming units (88%) compared to itraconazole alone. This study identified that saquinavir exhibits a potent synergistic relationship in combination with itraconazole against *Candida* species, which warrants further consideration.

KEYWORDS
Candida infections, saquinavir, azole resistance, efflux pumps, *C. auris* mouse model.

DISCOVERING THE ROLE OF LEUCINE-RESPONSIVE REGULATORY PROTEIN (Lrp) DURING CORN XYLEM INFECTION

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The bacterium *Pantoea stewartii* subsp. *stewartii* (*Pss*) causes Stewart's wilt disease in corn, an important agricultural commodity. *Pss* is introduced into corn via the corn flea beetle vector (*Chaetocnema pulicaria*) when its feces enter wounds created during feeding. The infection begins in the apoplast of the leaf where *Pss* causes leaf blight. Subsequently, the bacteria move to the xylem and form a biofilm, preventing water transport. This causes plant wilting and leads to necrosis, consequently affecting both crop yield and survival. A previous RNA-Seq analysis provided a snapshot of the *Pss* transcriptome during growth *in planta* and evidence of differential *Pss* gene expression when it resides in the xylem. Tn-Seq experimentation was subsequently used to identify specific genes essential to survival *in planta*. The current research project focuses on the physiological role of one of these essential genes, which encodes the global transcription factor, Leucine-responsive Regulatory Protein (Lrp). The Lrp protein family is found across the *Bacteria* and *Archaea*, where Lrp regulates multiple critical physiological functions. In *Pss*, Lrp is known to positively control motility and biofilm production, which are important for the *in planta* lifestyle of *Pss*. However, the full gene regulon controlled by *Pss* Lrp is unknown. By better understanding Lrp function, insights can be gained about how *Pss* recognizes and exploits the host environment. Current research concentrates on Lrp regulation of nutrient acquisition and metabolism when *Pss* resides in the corn xylem. Bioinformatic analysis of newly acquired RNA-Seq data comparing wild-type *Pss* to a Δ*lrp* strain has identified key genes, both activated and repressed by Lrp, including genes involved in controlling capsule biosynthesis and nitrogen-associated assimilation and metabolism. The RNA-Seq analysis results have been validated using DESeq2 bioinformatic analyses and qRT-PCR experimental analyses. Future efforts are aimed at purifying Lrp and determining its responsiveness to key xylem metabolites to better understand the *in planta* lifestyle of *Pss* as a model for other xylem-dwelling pathogens.

Transmission of La Crosse Virus in *Ae. triseriatus,* the Native Vector, and *Ae. albopictus*, an Invasive Candidate Vector.

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La Crosse virus (LACV) is the mosquito-borne causative agent of La Crosse Encephalitis, the leading arboviral cause of pediatric encephalitis in the US. LACV primarily affects children under the age of 16 and mild cases are febrile, but severe cases manifest with neurological dysfunction, coma, paralysis, seizures, and death. Historically, LACV comprised two lineages: lineage I in the Midwest and Appalachians and lineage II in the South, both of which cause human disease. Lineage III was first isolated in the Northeast in 2005 but has not been implicated in human disease despite isolation from mosquitoes. One potential explanation for this discrepancy is that lineage III is maintained vertically and subsequently not transmitted to humans through horizontal transmission. Elsewhere lineage I and II LACV are transmitted horizontally, between adult mosquitoes using reservoir species, and vertically, from parent to offspring. LACV is primarily transmitted by the native mosquito, *Ae. triseriatus*, but *Ae. albopictus*, an invasive mosquito, has been implicated in LACV transmission. Here we examined the differences in horizontal and vertical transmission among lineages in both species. For horizontal transmission, experimentally infected females were incubated for 14 days, and then viral presence in the body, legs, and saliva were used as indications of infection, dissemination, and ability to transmit, respectively. For vertical transmission, experimentally infected mosquitoes were allowed to oviposit and the resulting offspring were screened for viral presence. We show a varied ability to transmit horizontally and vertically among lineages and within lineage III strains. Additionally, we explored the role of geographic matching of mosquito species and virus origin on transmission and demonstrated a similar pattern with some virus strains capable of increased transmission when geographically matched to local mosquitoes but others with decreased transmission. Overall, this data indicates that within-lineage LACV strains may differ phenotypically.

Unpeeling the Layers of Lysophospholipid Metabolism in *Plasmodium falciparum*-Infected Erythrocytes

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Lysophosphatidylcholine (LPC) is an abundant serum lipid class that provides essential fatty acids and choline for *Plasmodium falciparum* lipid synthesis, yet the enzymes responsible for the hydrolysis of exogenous LPC are unknown. Using a novel *in situ* assay along with inhibitor profiling, we found that the relevant lysophospholipases belong to the serine hydrolase superfamily. Generation of transgenic parasite lines deficient in up to four candidate enzymes revealed that the loss of two of these, exported lipase (XL) 2 and exported lipase homolog (XLH) 4, dramatically reduced parasite lysophospholipase activity *in situ* in infected erythrocytes and in *in vitro* assays, and sensitized parasites to LPC toxicity. While XL2 was exported to the host cell, XLH4 was retained within the parasite, revealing a functional, but not spatial, redundancy in LPC metabolism. Knockdown of an additional parasite serine hydrolase ("prodrug activating and resistance esterase", PARE) on a quadruple-knockout background further reduced *in situ* LPC hydrolysis and abolished parasite replication in medium containing LPC as sole fatty acid source. In addition, parasites deficient in LPC metabolism were unable to proliferate when cultured with human serum, a physiologically-relevant fatty acid source that contains abundant LPC. Wild-type parasites were sensitized to a lysophospholipase inhibitor when cultured in serum, reinforcing the importance of these enzymes for efficient growth in high-LPC environments and establishing the feasibility of targeting LPC metabolism.

RIFT VALLEY FEVER VIRUS L POLYMERASE PHOSPHORYLATION REGULATES VIRAL RNA PRODUCTION IN A PROTEIN PHOSPHATASE-1 MANNER

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Rift Valley Fever Virus (RVFV) is an arthropod born negative sense RNA virus from the genus *phlebovirus*, family Phenuiviridae. Known for most commonly causing mild to moderate febrile illness in humans, RVFV can also progress to hemorrhagic liver necrosis and encephalitis in a smaller percentage of cases. RVFV represents an important pathogen of interest for research due to a lack of preventative measures and treatments, combined to its rising threat to public health and agriculture, causing a phenomenon described as abortion storms in ruminants. RVFV has been categorized as a Category A priority pathogen by the CDC and USDA due to its ease of aerosolization and host susceptibility.

Protein phosphatase 1 alpha (PP1α) is a very versatile serine/threonine host phosphatase protein known to be involved in the regulation of several cell mechanisms, such as cell cycle, mRNA splicing, and transcription. Cell regulation by PP1α occurs through interaction with other proteins via its various binding motifs, more importantly the RVxF binding motif. Previous studies have demonstrated that many viruses bind to and make use of PP1α as part of their replication process. Our studies have identified two putative RVxF binding motifs within the RVFV viral RNA-dependent RNA polymerase (L protein) in addition to confirming the binding of PP1α and the L-protein upon co-immunoprecipitation assays. Additionally, a prominent role of PP1α in RVFV replication has been discovered, in which a knockdown of PP1α resulted in decreased viral titers early in the infection. With that in mind we hypothesize that PP1α binds to L polymerase through its RVxF binding motif, regulating L protein phosphorylation and promoting viral replication. These newly found RVxF binding motifs have since been mutated within the genome of RVFV. Our studies aim to make use of the RVxF mutants to further investigate the importance of the L-polymerase interaction with PP1α for viral replication, in addition to exploring differences in viral kinetics upon mutation of the binding region.

Immunology & Host-Pathogen Interactions
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Model To Study Impact Of Gut Phages On Host Health

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The gut microbiome includes a rich community of viruses that includes an abundance of phages. This composition of this viral community is correlated with various GI diseases, though the extent of their causal impact remains unclear. To better study the impact of phages on the bacterial community and mammalian host, we developed a method to effectively reduce the viral particles from a murine microbiome with minimal impact on bacterial density. This treatment can be followed by a viral reconstitution returning the virome to its previous pre-treated state allowing to study the impact of perturbations on the microbiome in the presence and absence of phage. We have found that the presence or absence of phages in the gut microbiome alters the effect of diet and antibiotic response in murine models of the gut microbiome.

Multilocus Sequence Analysis Reveals Six *Fusarium* Species Complexes Associated with the Outbreak of Fusarium Wilt in Flue-Cured Tobacco

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In 2022, an outbreak of Fusarium wilt caused significant losses in flue-cured tobacco in the Piedmont Regions of Virginia and North Carolina. To characterize the diversity of *Fusarium* species associated with the disease, leading edges of symptomatic flue-cured tobacco plant tissues were surface sterilized and cultured on potato dextrose agar medium (PDA) to obtain *Fusarium* isolates. Fifteen isolates with distinct colony morphology were selected for molecular identification by comparing sequences of the internal transcribed spacer (ITS) against the Fusarium-ID database. Subsequently, a multilocus sequence analysis (MLSA) using the concatenated sequences of three housekeeping genes, translation elongation factor-1 alpha (*tef1*), the largest subunit of RNA polymerase II (*rpb1*), and the second largest subunit of RNA polymerase (*rpb2*), revealed that the fifteen isolates representing six *Fusarium* species complexes. Among these complexes, the *Fusarium incarnatum-equiseti* species complex was the most prevalent (33%), followed by the *Fusarium oxysporum* species complex (FOSC) (20%), *Fusarium solani* species complex (20%), *Fusarium fujikuroi* species complex (13%), *Fusarium sambucinum* species complex (7%), and the *Fusarium tricinctum* species complex (7%). To assess the pathogenicity of each isolate, pot experiments were conducted in a greenhouse using a flue-cured tobacco cultivar 'K326'. All inoculated plants showed increased leaf necrosis and decreased stem diameter, dry root weight, and dry shoot weight compared to the control. We are using the MinION nanopore sequencing platform to obtain the whole genome of each *Fusarium* isolate for comparative genomics analysis to further our understanding of their virulence mechanisms.

Effects Of Cold Temperatures On Avian Immune Response To A Bacterial Pathogen

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The host immune response to an invading pathogen can be highly variable among individuals, resulting in heterogeneity in both infection and disease severity. Understanding such heterogeneity is key because it can influence the likelihood of disease outbreaks in a host population. For endothermic animals, energetic constraints at subthermoneutral temperatures may alter both the magnitude and variability of host immune responses, but the effect of ambient temperature on immune variability has not been directly tested. In a world facing increased frequency of cold and hot spells, understanding the effect of subthermoneutral temperatures on immune responses is critical. I used temperature-controlled chambers housing wild-caught house finches to test this hypothesis. House finches were held in rooms at sub-thermoneutral (4-9ºC night-day) or thermoneutral (22-27ºC night-day) ambient temperatures. Half of the birds at each temperature were infected with the bacterial pathogen *Mycoplasma gallisepticum*, and several measures of host immune responses were quantified over the course of infection. To robustly quantify the immune response, whole body responses, as well as aspects of cellular and humoral immunity were measured, including conjunctivitis severity, mass, fever, phagocytic activity, haptoglobin, compliment mediated lysis, natural antibodies, and anti-MG antibodies. Here I present preliminary analyses testing how ambient temperature, MG infection, and their interaction influence the mean and degree of variation in immune responses between treatment groups. While immune assays are ongoing, we predict that mean immune responses will be depressed and overall responses will be more variable in hosts housed at subthermoneutral versus thermoneutral temperatures.

A Genome Similarity-Based Taxonomy Could Have Provided Immediate and Stable Identifiers For The SARS-CoV-2 Species And Its Lineages

When SARS-CoV-2 emerged in December 2019, it was referred to as nCOV19. The species name SARS-CoV-2 was only validly published in April, 2020. Therefore, the first reports about SARS-CoV-2 cannot be found by searching for "SARS-CoV-2". When the first variants of SARS-CoV-2 emerged, within-species classification and nomenclature systems were not in place leading to multiple names for the same variants creating unnecessary confusion. Over time, new systems were proposed leading to additional names creating more confusion. We asked the question if the previously proposed genome similarity-based Life Identification Number (LIN) approach could have been used to provide a stable species identifier as soon as the first SARS-COV-2 genome was released and if LINs could have been used to provide unique names to lineages and variants/clades as they emerged. Since genome similarity-based approaches had not been used for SARS-CoV-2 classification before, it was first necessary to determine to what extent Jaccard distance trees correlate with phylogenetic trees of SARS-CoV-2 sequences. To test the correlation between trees, 679 SARS-CoV-2 sequences belonging to eight clades/variants (G, GH, GK, GR, GRA, GRY, GV, and S) and 20 reference sequences (including RaTG13, the closest known relative of SARS-CoV-2) with metadata were downloaded from the GISAID and NCBI databases. These viral sequences were analyzed by both, the kmer-based sourmash tool to compute Jaccard distances and the software IQ-tree to build phylogenetic trees, and the resulting trees were compared by constructing tanglegrams. Consistent correlation between distance trees and phylogenetic trees gave the first indication that sourmash could be used for LIN assignment of SARS-CoV-2 genomes. LINs were then assigned sequentially to viral sequences in order of isolation date. We found that the assigned LINs would have been useful in classifying SARS-CoV-2 sequences as a new species and would have provided a LIN as a unique and informative identifier as soon as the first SARS-CoV-2 sequence was obtained. LINs also showed agreement with phylogeny-based lineages. However, LINs rarely had a one-to-one relationship with lineages. Thus, multiple LINs would have been needed to delineate each lineage. For naming lineages, however, the LIN assigned to the first sequence of a new lineage could still have provided a unique lineage name. Further research is being conducted to investigate whether distance and phylogenetic trees group sequences into similar clades at the variant level and if protein-based k-mer distances could improve reflection of phylogenetic relationships by distance trees and provide LINs that more accurately reflect those relationships.

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Multiscale Models of Usutu virus infection and transmission

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Usutu virus is a mosquito-borne flavivirus maintained in wild bird populations, with occasional spillover in humans. Understanding USUV epidemiology requires characterization of host-virus ecology at many scales including individual avian host infections, bird-to-vector transmissions, and USUV incidence in bird and vector populations. To provide insight into the intrinsic complexity of host-virus processes in individual birds, we developed a within-host mathematical model of Usutu virus infection from chicken host data taking into consideration variations in virus strain and host immune competency. We investigate transmission and incidence predictions based on laboratory-type inoculation and transmission experiments combined with dynamical mathematical modeling. We developed a multiscale vector-borne epidemiological model of Usutu virus infection in birds and mosquitoes and used individual within-host viral load data from sparrows and host-to-vector probability of transmission data to predict USUV incidence in bird and mosquito populations exposed to two different Usutu viral strains. Within-host we observed differences in virulence amongst virus strains, and found immune priming coupled with competent response leads to control of viral spread. Through our multiscale approach we found within-host peak viremia does not always correlate with infection incidence levels in host and vector populations, and that uncertainty in predictions at one scale may change predicted results at another scale. We demonstrated that optimal experimental design and increased frequency of data collection vastly improve the characterization of virus dynamics and transmission probabilities. These results may be useful for predicting spillover events.

Temperature Sensitive Nix: A Temperature-Dependent Method for Sex Separation of the Yellow Fever Mosquito *Aedes aegypti*

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Aedes aegypti female mosquitos are responsible for the spread of multiple arboviruses causing deadly diseases such as Zika, dengue, yellow fever, and chikungunya. Current methods preventing the spread of disease primarily include controlling mosquito populations using insecticides, however rising insecticide resistance threatens the method's efficacy. Genetic control strategies such as sterile insect technique (SIT) and incompatible insect technique (IIT) are promising methods of *A. aegypti* population suppression via mass release of infertile male mosquitos. However, current methods of rearing and separating mosquitos to obtain infertile males are expensive and laborious bottlenecks in these programs. Thus, it is crucial to develop innovative and effective methods for sex separation. Recently, the male-determining factor of *A. aegypti* was identified as a gene called *Nix*. NIX expression in genotypic females is enough for conversion to fertile, albeit flightless, males. We computationally determined residues in the NIX protein most likely to confer temperature sensitivity when mutated, and successfully created two *A. aegypti* lines that demonstrate temperature sensitive function of NIX (TSNix). We established a line of mosquitos where both male and female genotypes contained *TSNix* and by changing the rearing temperature we can manipulate sex determination in the genetic females. When reared at 25 °C, only males were observed, indicating TSNIX was functional in genotypic females and thus caused conversion of females to flightless males. When reared at 32 °C, a small number of flying and fertile TSNix-containing females were found. The absence of females at the permissive temperature of 25 °C and the presence of females at 32 °C indicates the function of TSNIX at the restrictive temperature was, to some extent, affected by the mutation. These lines can be used to rear all-males in the laboratory by only changing the rearing temperature, creating an effective and simple method of all-male production for mass release programs.

Confronting Antimicrobial Resistance: Nitroxoline's Promise in Gonorrhea

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Abstract

Gonorrhea is a sexually transmitted disease caused by *Neisseria gonorrhoeae*. It ranks as the second most common sexually transmitted bacterial infection in the United States. The World Health Organization (WHO) reports that about 2 out of 100 people worldwide are infected with gonorrhea. The surge of *N. gonorrhoeae* infections has been attributed to the emergence of multidrug-resistant strains of this bacterium. *N. gonorrhoeae* has developed resistance to every single class of antibiotics, leaving the medical community with limited treatment options. Currently, ceftriaxone remains the sole recommended antibiotic for treatment of *N. gonorrhoeae* infections; however, high-level resistance to ceftriaxone is growing concern. This raises the alarming prospect of an impending era in which gonorrhea becomes untreatable. Therefore, there is an urgent need for the development of novel anti-*N. gonorrhoeae* agents. Utilizing a drug repurposing strategy, we identified the FDA-approved drug, nitroxoline as a potent inhibitor for *N. gonorrhoeae.* Nitroxoline possesses multiple desirable attributes as a promising anti-gonococcal therapeutic. It is a broad-spectrum agent against urinary tract infections. Consequently, nitroxoline can accumulate at a sufficient concentration at the site of gonococcal infection (urogenital tract) to clear the infection effectively. Importantly, nitroxoline demonstrates minimal toxicity and has a well-established therapeutic history with no reported instances of increased resistance. Accordingly, we evaluated its anti-gonococcal activity against a panel of clinically important multidrug-resistant *N. gonorrhoeae* strains including the azithromycin- and ceftriaxone-resistant strains. The drug displayed a potent activity inhibiting 90% of the strains tested (MIC_{90}) at the concentration of 0.5 µg/mL. Time-kill experiments demonstrated a significant reduction in the growth of ceftriaxone-resistant *N. gonorrhoeae* strains within 14 hours of exposure to nitroxoline. Nitroxoline effectively penetrated human cervical adenocarcinoma cells, eradicating intracellular *N. gonorrhoeae*. Furthermore, nitroxoline exhibited minimal antibacterial effects on all tested species of commensal lactobacilli. These findings also suggest a low likelihood of *N. gonorrhoeae* developing resistance to nitroxoline. In conclusion, the repurposing of nitroxoline, an FDA-approved drug with a favorable safety profile and demonstrated effectiveness against multidrug-resistant strains, offers a promising avenue for tackling *N. gonorrhoeae* infections. Further investigations are warranted to fully elucidate nitroxoline's potential as a novel anti-gonococcal therapeutic.

NATURALLY OCCURRING SEX-LINKED RECESSIVE LETHAL ALLELES (RLAs) AND SEX RATIO DISTORTION IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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Abstract

Aedes aegypti female mosquitoes are critical public health issues on a global scale, as they are vectors of many arboviral diseases, such as Zika, Yellow fever, Dengue, and Chikungunya viruses. Currently, preventative strategies depend on effective vector control, which is hindered by the increasing insecticide resistance. There have been several research efforts, such as using genetic tools for population suppression and population modification—by reducing the female population or making the females resistant to virus transmission, respectively. Several workers have reported the presence of recessive lethal alleles (RLAs) on the Y-like chromosome of *A. aegypti* that result in sex ratio distortion. Here, we report the discovery of naturally-occuring RLAs on the X-like chromosome. To map these novel RLAs, we generated several genetic strains that will enable rapid identification of the RLA loci through marker-assisted mapping. Upon successful completion, this study will provide insights into the evolutionary forces that led to the occurrence and persistence of sex-linked RLAs. The study could also help remove the biting females so only males are released in the previously described genetic control programs. In the long run, the outcome of this study would serve as part of the toolbox for effectively preventing *A. aegypti*-borne infectious diseases, improving human health and protecting millions of lives worldwide.

Impact Of Brewing Industry Byproducts Used As Feed Additives On Aquaculture-Raised Rainbow Trout (*Oncorhynchus mykiss*) Under Thermal Stress Conditions

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The aquaculture industry is an essential alternative to wild-caught fisheries and is used to meet the increased demands of feeding seafood to a rapidly growing human population. To increase the sustainability of aquaculture practices, a collaboration was formed with Anheuser-Busch (Switzerland) of the beer brewing industry and Maltento (South Africa), a functional ingredient company. The two largest byproducts of the beer brewing process are brewer's spent yeast (BSY) and brewer's spent grain (BSG). These low-value waste products were converted into high-value feed additives used in the diets of aquaculture-raised rainbow trout (*Oncorhynchus mykiss*). A seven-week trial was conducted in a recirculating aquaculture system using 24 tanks, with each treatment replicated four times at the tank level with 12 fish stocked per tank. A commercial feed, Ziegler Bros., Finfish Silver Semi-floating was used as the control diet. BSY was processed enzymatically and coated onto the commercial feed at a 2% concentration (BSY1) and a 0.75% concentration (BSY2). In addition, insect products were obtained by culturing black soldier fly larvae on BSG. The insect diets were coated with two different products, denoted BSF3 (from a previous trial) and BSF4, at a 3% and 0.75% concentration, respectively. Lastly, a sixth diet was comprised of a combination of BSY2 and the BSF4 insect product top coated at a 1.5% concentration (mixture at 0.75% for both of the diets, 1.5% total). Various fish physiological parameters were monitored to observe the impact of the experimental diets on the rainbow trout. Variables included survival rates, growth rates, feed efficiency, biometrics, and bacterial community sampling of the fish. To test for possible shifts in the bacterial community, DNA samples were purified from the intestinal tract of the rainbow trout, and the V4 region of the bacterial 16S rRNA gene was amplified and sequenced. Due to a malfunction in the chiller used to maintain cool water temperatures, temperature and dissolved oxygen became substantial stress factors near the end of the trial. Preliminary results suggest that fish fed the experimental diets benefitted by having higher survival rates.

TOWARD QTL MAPPING OF HYBRID MALE STERILITY IN THE ANOPHELES GAMBIAE COMPLEX MOSQUITOES

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Abstract

Malaria is a life-threatening disease that is caused by parasites and transmitted by mosquitoes. In 2021, there were 247 million cases of malaria and hundreds-thousands of deaths reported by the WHO. ith the ecological problems caused by use of pesticides, and growing resistance within malaria vectors, genetic approach starts to draw more attention in controlling population of malaria vectors. As the main malaria vector, the *Anopheles gambiae* complex consists of 8 species that started diverging about 0.5 million years ago. Offsprings from the crosses of these species can suffer serious reduction of fitness, specifically hybrid male sterility, which is caused by failure in pre meiosis and meiosis and lead to abnormal and undeveloped sperm. However, the genetic basis for failure is unknown and our study aims at mapping genetic factors. In the hybrid offspring of male *Anopheles coluzzii* MOPTI and female *Anopheles merus* MAF, females were taken and backcrossed with *Anopheles merus* males, the offspring was named BC1. BC1 has heterozygous genomic regions from *coluzzii* with heterozygous and homozygous *merus* genetic material. BC1 gains partial sterility in males. So far, 26 BC1 were collected, dissected, and categorized with different phenotypes of spermatozoa, then the carcasses were saved for whole genome sequencing and association with phenotype categories. We observed six phenotypes in the following percentages. 1) Normal testes with bundles of normal sperm (3.8%). 2) Normal-like testes with mostly small-head sperm (19.2%). 3) Dysgenic testes with mostly large-head sperm (42.3%). 4) Dysgenic testes with only abnormal large-head sperm and short tails (23.1%), which is the phenotype observed in F1 males from the ♀*A. merus* / ♂*A. coluzzii* cross. Studying the cellular and genetic mechanism of hybrid male sterility becomes critical toward understanding the reproductive processes and can be used to control populations of mosquitoes.

Krisangel López

Title: MOSQUITO AND VIRAL DIVERSITY ACROSS VIRGINIA

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An understanding of pathogen incidence, prevalence, distribution, dispersal mechanisms, transmission dynamics, and predictive factors that increase risk of emergence, is critical for developing effective biodefense strategies. We surveyed three sites across Virginia: including Coastal Plain (swamp), Piedmont (savannah), Blue Ridge (primary forest) for one week per month from early May until early October in 2022. Mosquitoes were collected and sorted into pools by species based on morphological identification. Pools were also screened for potential virus via cytopathic effect assays using three different cell lines (Vero-76, BHK-21, and C7/10) at three different temperatures, 37° C, 30° C, and 28° C, respectively. A total of the 7899 mosquitoes were collected and sorted into 971 pools, of which 638 have been screened. So far there are 217 potential and 25 confirmed positives, including 14 flaviviruses, and 9 bunyaviruses. Mosquito pools will continue be screened throughout September and various known and novel viruses will be sent off for genetic sequencing. As of now, we have identified four insect-specific flaviviruses of which will be aligned and characterized in the upcoming months.

Using PSO-GA and Virtual Screening Methods to Efficiently Develop Antivirals for Proteases in Dengue and Chikungunya Viruses

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Positive-sense RNA viruses have a high rate of genetic recombination, which poses a significant challenge to drug discovery efforts. Positive-sense RNA viruses including Dengue and Chikungunya viruses are global health threats that highlight a need for more efficient drug discovery methods, as well as the development of broad-spectrum antivirals to withstand the elevated rate of mutations. The integration of novel methodologies to functionalize and repurpose compounds with computational methods including virtual screening have been shown to increase the efficiency of the drug design process. Refining and elevating these screening methods and algorithms also requires a diverse set of structures to screen against to increase the potential success of computational hits. Proteases have emerged as a promising target for broad-spectrum antivirals due to their highly conserved catalytic site and crucial involvement in the replication and pathogenicity of RNA viruses. Considering the deficiency of structural data for these proteases, this study aims to investigate the efficacy of molecular-dynamics-generated structures for drug design. Molecular dynamics simulations performed on the crystal structure of the NS2B/NS3 Dengue virus protease complex (PDB ID 2FOM) highlight the dynamic behavior of the NS2B cofactor. During simulation, the NS2B cofactor did not transition to its active conformation, limiting the set of conformers for subsequent screening and mechanistic insight. Root Mean Square Fluctuations (RMSF) for each individual residue reveal an important dynamic area in the protease involving the residues present in the two β-hairpins of the NS3 protein proximal to the catalytic residues where the cofactor should hypothetically anchor itself between in an active conformation. Future directions include molecular dynamics simulations with benzoyl-norleucine-Lys-Arg-Arg-aldehyde, a covalently bound peptide, to elucidate the mechanism of action of the NS2B/NS3 protease complex.

INVESTIGATING THE DAILY RHYTHMS OF HUMAN ODOR AND MOSQUITO OLFACTION

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Biological rhythms are critical to the survival and health of all animals, including insects, as they enable organisms to predict and synchronize with environmental cues, optimize physiological processes, and coordinate behavior based on the timing of key events. Among insects, mosquitoes time their host-seeking behavior to optimize their chances of obtaining a blood meal, without being killed in the attempt. While the importance of olfaction in mediated mosquito-host interactions has been established, the chemical rhythms underlying these interactions are yet to be characterized. This knowledge gap is particularly relevant for species such as Aedes aegypti, which are highly anthropophilic and can transmit a variety of pathogens to humans. In this context, we analyzed how daily rhythms in human scent align with mosquito activity patterns. We collected human odor samples at multiple times of the day and identified daily changes in the chemical composition of human odor. We leveraged this knowledge to create artificial human odor mixtures, and used behavioral methods to determine the relationship between daily rhythms in human odors and mosquito attraction. We found significant differences in human odor at different time points, and created artificial odor mixtures specifically by identifying the chemicals that were statistically significant between the 6 AM and 6 PM time points. These mixtures can represent human odors at different time points and can be used in cage landing experiments to study the impact of human odor rhythms on the behavioral preference of mosquitoes. Our findings have important implications for understanding the underlying mechanisms governing mosquito-host interactions, and open new research avenues for the development of control strategies.

Impact of Lysis-Lysogeny Decision-Making on Bacterial Resistance to Temperate Phages.

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Amid the increasing interest in harnessing phages for therapeutic applications against bacterial infections and capitalizing on their potential to manipulate bacterial hosts through predation and lysogeny, understanding the mechanics of phage infection and shifts in bacterial fitness dynamics becomes essential. Temperate phages can pursue both lytic and lysogenic life cycles. In response, bacteria employ diverse strategies to develop resistance against phages, including altering surface receptors, degrading phage DNA, and inhibiting DNA injection.
While the benefits of protective mechanisms against virulent phages are clear, the fundamental drivers and rationale behind resistance emergence to temperate phages remain insufficiently explored. This study aims to explore the factors driving resistance development to temperate phages.
We generated P22 phage mutants and observed that when exposed to *Salmonella enterica* Serovar Typhimurium (STm), lysogeny was prevalent, leading to reduced phage resistance similar to wildtype P22 phage effects. Intriguingly, P22 mutants lacking the *immI* region, which initiates the lytic cycle by deactivating the C2 repressor, exhibited higher phage resistance and lower lysogeny. Deleting the P22 *immI* region didn't hinder lysogen growth or phage production. Even in competition with P22-resistant bacteria, deleting the P22 *immI* region didn't compromise lysogen fitness. In fact, these lysogens showed greater fitness than P22-resistant STm, similar to other lysogens retaining the *immI* region. This research highlights that the *immI* region's absence reduces lysogeny frequency per infectious phage, ultimately promoting the enrichment of P22-resistant bacterial strains.

Title: DETERMINING THE ROLE OF A NOVEL TRANSCRIPTION FACTOR TgAP2X-7 IN *TOXOPLASMA* BIOLOGY AND PATHOGENESIS.

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Abstract:

Toxoplasma gondii is a protozoan parasite that is identified as a primary cause of miscarriage in pregnant women, blindness in newborn children and fatal encephalitis in immunocompromised individuals. *Toxoplasma* follows an indirect mode of life cycle with cats acting as definitive host and a large number of livestock serving as intermediate hosts. Humans acquire *Toxoplasma* infection by multiple routes including ingestion of contaminated food or water, ingestion of contaminated meat and transplacental transmission during pregnancy. In humans, *Toxoplasma* replicates intracellularly through an asexual method termed endodyogeny, and the emergence of new daughter parasites leads to the destruction of the host cell. The pathology of toxoplasmosis is attributed to the lytic mode of propagation. Hence identifying unique parasite factors associated with various stages of the lytic cycle, such as invasion, replication, and egress, is crucial for the development of novel therapeutics. ApiAP2s are a family of transcription factors in *Toxoplasma* that are quite divergent from the transcription factors seen in higher eukaryotes and hence are considered good drug targets. We are specifically focusing on TgAP2X-7, a transcription factor whose function is yet to be determined, but has been predicted to be essential for *Toxoplasma* propagation. To determine the function of TgAP2X-7, we took a conditional knockdown approach using the recently developed AID (Auxin Inducible Degron) system. Accordingly, we successfully inserted the mAID HA epitope tag at the C-terminus of the gene using CRISPR-Cas9 technology. Immunofluorescence analysis (IFA) demonstrated that the protein resides in the parasite nucleus. Plaque assays suggested that without this protein, the *Toxoplasma* propagation *in vitro* is completely abrogated. The absence of the TgAP2X-7 results in slower rate of parasite replication and importantly, significant defects in host-cell invasion. Our future objectives include determining changes in gene expression, dissecting the role of different protein domains, identifying the interactome, and deciphering the signalling pathways that dictate the function of this novel transcription factor in parasite gene regulation.

Synthesizing Modular Hyperbranched Glycomaterials With Terminal Sialic Acid Or Mannose Moieties For Inhibiting Influenza Infections

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Abstract Text:

Influenza, a large endemic family of viruses, comes with a significant health burden—hundreds of thousands of infections and tens of thousands of deaths occur annually in the United States alone. Though antiviral medications and vaccines are utilized to help control outbreaks, the virus's ability to easily spread enables its constant mutation. This ease of variation can frequently make current treatment or control strategies ineffective or challenging. One supplement to current antiviral approaches is multivalent glycopolymers, a class of antiviral materials. In nature, biological entities commonly utilize multivalent interactions to enhance binding to other biological surfaces (e.g., receptors on the viral surface binding to sugars on a cell surface). Sialic acids and mannoses are used in this study as influenza is well-known to bind to these sugars in the lungs. Here, we report the synthesis of modular hyperbranched polymers using reversible addition-fragmentation chain-transfer (RAFT) polymerization of tetrafluorophenyl acrylates. These polymers contain trithiocarbonate terminal ends and an activated ester along the polymer chain, allowing for selective and controlled post-polymerization modifications at both the repeat unit and terminal end groups. By changing the molecular weight or degree of branching of the polymer and generating a large library of systematically varied materials, this study investigates the role of the polymer's architecture on influenza inhibition. The antiviral efficacy of these glycomaterials will be analyzed with a hemagglutination inhibition assay. We aim to elucidate structure-property relationships that will allow us to better maximize antiviral inhibition through material design.

Development Of Experimental Tools for the high-throughput Analysis of *Aedes aegypti* Activity And Posture

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Mosquitoes transmit numerous human pathogens, such as the dengue virus and *Plasmodium falciparum*, responsible for nearly one million yearly deaths. In addition to rising insecticide resistance, current control strategies are being confounded by high levels of mosquito behavioral plasticity. In particular, biological rhythms are crucial to disease transmission as they allow mosquitoes to be active when their hosts are available and vulnerable. However, despite clear epidemiological relevance, a lack of experimental tools has prevented in-depth analyses at various biological scales. For example, at larger scales, the activity patterns of groups of mosquitoes are typically tracked under controlled laboratory settings that require costly equipment. At smaller scales, tracking individual behavioral features typically requires a significant time investment by the experimenter. In this context, we built off of existing solutions (PLAM) and engineered a *Raspberry Pi*-based rig, programmed with custom motion-tracking scripts that record the diel activity patterns of groups of mosquitoes. Our results show activity patterns similar to validated patterns from prior studies, making our system ready for field deployment. At the individual scale, *Aedes aegypti* mosquitoes are known to exhibit unique postural changes upon entering a sleep-like state. But because of the tedious nature of hand-labeling data, mosquito sleep remains largely understudied. Here, we used "SLEAP", an open-source deep-learning-based framework for multi-animal pose tracking, to accurately track the joints of mosquitoes as they enter their sleep-like state. Our trained models can be used for mass analysis, opening new research avenues to improve our understanding of this facet of mosquito chronobiology.

Antimicrobial Resistance in Commensal *Escherichia coli* and Other Bacteria in Small Ruminants

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Introduction The emergence of antimicrobial resistance (AMR) is a phenomenon important to both human and animal health globally. The Center for Disease Control and Prevention (CDC) reports that over 2.8 million people acquire an antibiotic-resistant infection each year in the US with over 35,000 deaths occurring as a result of these infections. Overuse of antibiotics, e.g., inappropriate prescribing of antibiotics and extensive use of antibiotics in animal agriculture, is suspected to contribute to the evolution and spread of resistance. Sheep and goats are an important source of meat and milk globally and are gaining popularity in North America as a meat source due to the influx of ethnic groups from areas of the world where goat meat consumption is common. Only five studies were found in the literature documenting foodborne pathogens in small ruminants in US farms.

Rationale and Significance: Data on the level of AMR and potential factors responsible for occurrence, acquisition and spread in US small ruminant production systems are lacking. The small nature of these systems may result in widespread antibiotic use practices.

Objectives 1. Determine knowledge, attitudes and behavior around use of antimicrobials by small ruminant farmers and veterinarians in Virginia**. 2** Quantify the prevalence and diversity of phenotypic and genotypic antibiotic resistance among commensal and pathogenic *E. coli* from small ruminants. **3** Quantify the Prevalence of *Campylobacter*, *Salmonella*, and their resistance profiles in small ruminants.

Hypotheses Veterinarians and farmers who are more educated/aware of the problem of AMR and likely contributing factors are more likely to participate in AMR mitigation efforts.

Research approach We recently developed a questionnaire that will be used to survey small ruminant farmers and veterinarians in Virginia regarding their knowledge about AMR and also their attitudes and practices surrounding antimicrobial use. In parallel with the survey, fecal samples have been collected from sheep and goat populations that have reported antimicrobial use and control populations. *E. coli* have been isolated and will be subject to Kirby-Bauer disk diffusion following recommendations by the Clinical and Laboratory Standards Institute to determine the phenotypic resistance profiles. Confirmed *E. coli*, *Salmonella* and *Campylobacter* isolates will be tested for their susceptibility to 12 antimicrobials agents that represent different antimicrobial groups of medical importance. Isolates with resistance phenotypes to any of the tested antibiotics will further be subject to whole genome sequencing in order to determine their genotypic resistance profiles. We will additionally analyze the fecal samples for antibiotics to determine if reported use is consistent with the antibiotic detected.

Expected results We expect to gain a better understanding of antimicrobial drug use practices for small ruminants plus the knowledge and perception of small ruminant farmers in VA. We expect

results that inform on the prevalence of AMR in commensal *E. coli* as well as pathogenic enteric bacteria (pathogenic *E. coli* serotypes, *Salmonella* and *Campylobacter*) and identification of genes responsible for resistant phenotypes in small ruminants in Virginia. The findings can help to provide farmers and veterinarians with more specific guidance regarding best practices for antimicrobial use.

Mapping Lyme Disease Exposure in Dogs: Insights from Companion Animal Clinic, Blacksburg, VA

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Lyme Disease presents a significant public health concern, not only for animals but also due to its potential spill over risk to humans. Despite this, a comprehensive understanding of the prevalence of Lyme Disease in dogs, particularly in specific geographic areas, remains limited. This presentation outlines a practicum-based study at the Companion Animal Clinic in Blacksburg, VA, aimed at addressing this knowledge gap. The primary objective was to analyze positive Lyme Disease test results from canine patients at the clinic, creating a geographical map highlighting potential exposure areas. This study investigated the correlation between exposure, preventative measures, and vaccination status. Data collection involved assessing the clinic's database to retrieve SNAP 4Dx tests and Quant C6 test results from 2021. Subsequent analysis and mapping were performed remotely using appropriate software tools. The goal being, to identify exposure patterns, aiding Companion Animal Clinic in educating clients about Lyme Disease risks and promoting preventative measures. Lyme Disease is the most common tick-borne disease in Virginia. This data-driven study contributes valuable insights into canine Lyme Disease exposure in the New River Valley area, providing essential information for both animal and human health management within the community.

Prior Exposure To A Pathogen Induces Heterogeneity In Susceptibility

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Individual variation in pathogen susceptibility can have key consequences for epidemiological dynamics. Prior exposure to pathogens often results in varying levels of protection when a host is faced with secondary exposure, and likely depends on the initial pathogen dose. However, the role of prior pathogen exposure in inducing this population-level heterogeneity in susceptibility remains largely unexplored. We use a natural system (house finches and a bacterial pathogen, *Mycoplasma gallisepticum* [MG]) to investigate how the degree of prior exposure to pathogens alters population-level host heterogeneity in susceptibility. To test how prior exposure to pathogens alters heterogeneity in susceptibility, we created variation in prior exposure by inoculating 157 house finches with one of three categorical treatments (sham, low, or high-dose MG exposure). Once birds recovered from prior exposure treatment (42 days post-inoculation), we assayed population-level heterogeneity using a dose-response approach by measuring infection success (susceptibility) of individuals exposed to five doses of MG. To assess heterogeneity in susceptibility, we fit dose-response models assuming heterogeneous or homogenous protection among hosts to quantify susceptibility. Prior exposure to MG resulted in lower mean susceptibility but higher heterogeneity in susceptibility in house finches. While birds with no prior exposure were well described by a model with homogenous susceptibility, estimates for population-level heterogeneity in susceptibility increased with the degree of prior exposure experienced (i.e., heterogeneity was greatest in birds with high-dose prior exposure). These patterns align with prior work on vaccination and heterogeneity in susceptibility and highlight the importance of prior pathogen exposure in driving population-level heterogeneity, and, subsequently, epidemiological dynamics.

Susceptibility of North American Avian and Mosquito Species to Usutu Virus

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Usutu virus (USUV) is an emerging mosquito-borne virus belonging to the *Flaviviridae* family and is known to cause neuroinvasive illness in wild birds and humans. It is maintained in an enzootic cycle primarily involving passerine spp. birds and *Culex* spp. mosquitoes. USUV was first isolated in South Africa in 1959 and has since spread throughout Africa and Europe, causing mass mortality of the Eurasian blackbird (*Turdus merdula*). In addition, there has been an increase in the number of human cases ranging from asymptomatic to neuroinvasive illness. To this end, we sought to investigate the susceptibility of various North American avian and vector species to USUV to better understand the maintenance and transmission of the virus. House finches (*Haemorhous mexicanus*) were trapped locally and inoculated with either USUV isolate Netherlands 2016 or Uganda 2012. HOFI were found to be susceptible to both strains of USUV with no significant difference in viremia for the two strains. HOFI reached viremias we would predict would be transmissible to mosquitoes. From initial serum samples, two HOFI were positive for West Nile virus antibodies, and these birds did not develop USUV viremia. To understand potential vector species competent for USUV transmission, we performed artificial infectious feeds with two *Culex* spp. mosquitoes, *Culex tarsalis* and *Culex pipiens*. Both species were weakly competent vectors, transmitting infectious virus at low levels in their saliva. However, for both species, there was no significant difference in infection rates for the two virus isolates. These results indicate avian and vector species that have the potential to be reservoir hosts and primary vectors of USUV and suggests that emergence of USUV in the United States is possible.

USING MOUSE CORONAVIRUS TO DETERMINE THE IMPACT OF OBESITY ON CORONAVIRUS DISEASE SEVERITY AND IDENTIFY BIOMARKERS OF SEVERE DISEASE OUTCOME

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Abstract

Coronavirus disease 2019 (COVID-19) has been shown to cause more severe or even fatal disease in the elderly and individuals with co-morbidities. Obesity is an important co-morbidity impacting ~13% adults globally and 43% adults in the U.S. and has been associated with increased risk of hospitalizations in COVID-19 patients. Current knowledge on the impact of co-morbidities is based on epidemiological evidence and/or infections of non-natural hosts with SARS-CoV-2, but COVID-19 disease outcome is determined by complex host-virus interactions including host's immune response to the virus. Therefore, a more relevant model is needed to establish a causal link between co-morbidities and coronavirus disease severity, which can then be used to better understand coronavirus pathogenesis and to identify biomarkers associated with severe disease outcomes. To that end, we infected lean and diet-induced obese (DIO) mice with mouse hepatitis virus (MHV)-1, a mouse coronavirus, and found that obese mice had more severe disease in terms of weight loss, mortality, and lung histopathology. To identify biomarkers of severe disease, we performed RNA sequencing (RNASeq) analysis on blood samples collected at 2 days-post-infection (dpi) and these can be used in timely diagnosis and risk stratification of patients for effective utilization of health care resources.

HOST-SEEKING NEURAL MECHANISMS OF *A. AEGYPTI* MOSQUITOES MODULATED BY INTRASPECIFIC LARVAL COMPETITION

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Aedes aegypti mosquitoes are one of the most invasive species and the deadliest disease vector, killing ~700,000 humans each year worldwide. *Ae. aegypti* adapt and thrive in diverse growing conditions and therefore are ubiquitous in distribution. They are the optimal vector for pathogens causing diseases such as the Zika, yellow fever, chikungunya, and dengue. Female mosquitoes use a variety of senses such as vision, hearing, thermosensation, and mechanosensation, besides chemosensation, to identify and seek hosts for blood-feeding. Mosquitoes' chemosensory perception, specifically in the context of host-seeking, is influenced by many extrinsic (e.g. environmental conditions) and intrinsic factors (e.g. mating status). Findings from behavioral assays on mosquito host-seeking behavior in the Vinauger lab reveal that competition for resources during the larval stages influenced the host preferences of adult females. Large-sized females from less competitive larval environments displayed a strong preference for odors from blood hosts than small-sized females from highly competitive larval environemnts. In this context, the goal of this research is to focus on the neural mechanisms that mediate the effect of adult body size on mosquitoes' olfactory sensitivity and perception of host odors. To address this objective, I performed electroantennogram experiments to quantify the sensitivity of large and small-sized mosquitoes to host odors at the peripheral level. Subsequetly, electrophysiological recordings have also been done to decipher how the representation of host odors in the mosquito antennal lobe is influenced by the presence and absence of carbon dioxide, a potent host cue. Research findings from this study bridges gaps in our mechanistic understanding of how larval ecology influence mosquitoes' interaction with human hosts and the ensuing disease consequences.

LANSOPRAZOLE INTERFERES WITH FUNGAL RESPIRATION AND ACT SYNERGISTICALLY WITH AMPHOTERICIN B AGAINST MULTI-DRUG RESISTANT *CANDIDA AURIS*

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Abstract

Candida auris has emerged as a problematic fungal pathogen associated with high morbidity and mortality. Amphotericin B (AmB) is the most effective antifungal used to treat invasive fungal candidiasis, with resistance rarely observed among clinical isolates. *C. auris* possesses extraordinary resistant profiles against all available antifungal drugs, including AmB. After screening a panel of ~800 FDA-approved drugs, we identified the proton pump inhibitor lansoprazole (LNP) as a potent enhancer of AmB's activity against *C. auris.* To deconvolute the LNP mode of action, we used the mass spectrometry-based Proteome Integral Solubility Alteration (PISA) assay to identify potential LNP-protein interactions. Subsequent experiments suggest that LNP interacts with an essential target in the mitochondrial cytochrome system complex III, (cytochrome bc1), increasing oxidative stress in fungal cells. The target was validated via transcriptome sequencing (RNA-Seq) analysis, cytochrome inhibitors rescue assays and utilization of a collection of deletion mutants. Our findings demonstrated the critical role of an active respiratory function in the antifungal activity of LNP. Most importantly, LNP restored the efficacy of AmB in an immunocompromised mouse model, resulting in a 1.7-log (~98%) CFU reduction in the burden of *C. auris* in the kidneys. In conclusion, we identified and validated that LNP could work synergistically with AmB against drug-resistant *C. auris* by targeting mitochondrial cytochrome bc1.

STRUCTURAL BASIS FOR TRANSMEMBRANE SIGNALING IN *P. AERUGINOSA* VIRULENCE REGULATION

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According to the CDC healthcare-associated infections rank among the leading causes of death in the United States, claiming 72,000 lives annually. Due to its propensity for developing multi-drug resistance the World Health Organization has declared the bacterium *Pseudomonas aeruginosa* a top priority pathogen. Already, *P. aeruginosa*-associated respiratory tract infections are the leading cause of mortality among people with cystic fibrosis, but it may also infect the skin, eyes, and the cardiovascular system. Acute and chronic *P.aeruginosa* infections require remarkably distinct repertoires of virulence factors. For example, the type three secretion system is critical for acute infections but dispensable during the chronic stage. Conversely, several exopolysaccharides are overproduced only to support the biofilm formation characteristic of the chronic stage. To coordinate the expression of these virulence mechanisms *P.aeruginosa*, like many other bacterial pathogens, relies on sensor histidine kinases (SK)-mediated phosphor-relay and two-component signaling systems. Despite their dominant role in bacterial signaling much remains to be learned about the signaling mechanisms of SKs. Traditionally these enzymes were thought operate exclusively along simple linear phosphor-transfer chains. Yet, in recent years number of multi kinase networks (MKNs) have been discovered, wherein crosstalk between SKs mediates co-regulation of distinct virulence determinants. Using the *P.aeruginosa* SK GacS as model system, we seek to elucidate molecular mechanisms underlying this crosstalk. The GacS/GacA phosphorelay, positioned at the heart of the perhaps most complex known MKN, upregulates virulence factors associated with biofilm formation and chronic infections. The SK family enzyme RetS directly inhibits GacS to promote the expression of virulence factors of acute infection, while suppressing those required for chronic disease. Important gaps remain in our understanding of how the activities of RetS and GacS are regulated through inter-extracellular signals. Both proteins feature essential periplasmic sensory domains, but the specific ligands remain unknown. Moreover, we recently uncovered that, mediated through their respective DHp domains, RetS and GacS form a domain-swapped oligomer. According to this partial structural model GacS will undergo a significant conformational transition upon RetS binding within its membrane-proximal HAMP domains. Because a close conformational coupling of HAMP, transmembrane helices and periplasmic sensory domains, we hypothesize that conformational switching within the cytosolic domains of GacS will also impact extracellular signal perception. Such inside-out signaling has not been observed among bacterial SKs and, if confirmed, would constitute a new paradigm for cross-membrane signaling for this family of enzymes. We propose to perform structural and biochemical studies to provide the first experimental evidence for bidirectional signaling in bacterial SKs.

Utilizing Reverse-Metabolomics to Explore Diet-Derived Microbial Metabolism

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The human metabolome, encompassing the systemic and circulatory molecules in the body that directly impact our health, is comprised of over 1.8 million metabolites, with the microbiome being responsible for producing the majority of these. As of 2022, only about 2% of these metabolites can be identified in any given untargeted human metabolomics study. To address the bottleneck of identification to MS spectral matches, a new method, "synthesis-based reverse-metabolomics," is utilized. Diet-derived phenolic acids and isothiocyanates, found in fruits and cruciferous vegetables, respectively, are large classes of molecules lauded for their health benefits. However, between individuals, purported health benefits may not be equal due to the variation of the intestinal microbiome that differentially metabolize diet-derived molecules. In this work, various phenolic acids and isothiocyanates are synthetically conjugated to a variety of proteinogenic and non-proteinogenic amino acids, capturing potential host and microbial metabolism that occurs in the body. Conjugates are then subjected to LC-MS, producing authentic MS/MS spectra that can be matched to unknown spectra in public data. This workflow aims to identify unknown diet-derived metabolites, as well as correlate metabolites with health-related phenotypes using sample metadata. Ultimately, our method will establish novel connections between microbiota-mediated metabolism of dietary compounds and health status at an inter-individual level.

Marcel Shams Eddin
CeZAP Symposium Abstract

Title: Characterization of Membrane Associated Proteolytic Cleavages Affecting Spore Germination in *Bacillus subtilis* and Possible Interacting Regions Between SleB and YpeB

Bacteria from the orders Bacillales and Clostridiales form spores that can persist for decades and can survive a wide range of killing mechanisms, including heat, desiccation, UV-radiation, and antibiotics. These spores can rapidly germinate in permissive environments and cause severe diseases like anthrax, tetanus, and food poisoning. SleB and CwlJ are the Germination Specific Lytic Enzymes (GSLEs) that degrade *Bacillus* spore peptidoglycan (PG) contributing to rehydration of the spore and transition to the vegetative state. SleB interacts with a partner protein, YpeB, which may hold it inactive until germination starts. The specific interaction between SleB and YpeB is unknown, thus we aim to use bioinformatic tools such as evolutionary covariation and molecular docking to understand this interaction. Homology modeling and preliminary AlphaFold multimer analyses suggest that YpeB and SleB form a higher order complex. To verify the interactions experimentally, site-specific mutagenesis will be used. YpeB is rapidly proteolyzed during spore germination, which may release SleB to degrade the PG. HtrC, a serine-type endopeptidase has been shown to cleave YpeB, but YpeB is still degraded in the absence of HtrC, showing that other proteases might be involved in the degradation process. Five putative proteases have been identified to be present in the spore inner membrane. We hypothesize that at least one of the five proteases will be serving HtrC's redundant role in YpeB degradation. A mutant strain lacking five proteases delayed germination for about 40 minutes when compared to WT. To understand whether YpeB cleavage is essential for SleB activity, YpeB stability will be studied through western blots. Identifying protein-protein interactions during germination could help us manipulate germination either by increasing the germination efficiency, thus rendering the spores more susceptible to killing, or by preventing germination, thus making the spores inefficient in causing human disease.

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SYNTHESIZING A LIBRARY OF SIALIC ACID-FUNCTIONALIZED POLYPEPTIDES FOR INFLUENZA INHIBITION

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Abstract

The recent pandemic has highlighted the need to continue investigating alternative methods for the inhibition of viral infections, especially in the early stages of an outbreak when more established methods (i.e., vaccines, antiviral medications) are unavailable or undeveloped. Influenza, a family of viruses that has historically produced endemic and pandemic outbreaks, is a ubiquitous infectious disease at the forefront of potential future outbreaks. Our research focuses on establishing an alternative antiviral approach using glycopolymers—synthetic polypeptides carrying pendant sialic acid moieties, a glycan well-known to bind to various influenza strains. By mimicking the polyvalent interaction between hemagglutinins (HAs) on the viral surface and sialic acids (SAs) on our cells' surface, we aim to design materials that influenza will preferentially bind to. Mimicking this interaction will prevent the virus from reaching the surface of our cells, hence preventing infection. Our investigations focus on how various structural properties of the polymer impact their ability to inhibit viral infections. We are systematically synthesizing a library of synthetic polypeptides with functionalized sialic acid pendant groups and changing polymer chain lengths, polymer conformation, and SA content. The antiviral efficacy of our glycopolymers will then be examined by hemagglutinin inhibition assays (HAI assays), elucidating fundamental structure-property relationships that will direct us toward maximizing antiviral inhibition.

Investigating the Immunogenicity of a Chimeric Zika Virus Vaccine Candidate

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Flaviviruses are positive sense RNA viruses that have caused epidemics worldwide since the 1600s. Pathogenic flaviviruses of medical importance include Yellow Fever, West Nile, Zika (ZIKV), Dengue, and Japanese Encephalitis viruses. Since its discovery in 1947, ZIKV has been responsible for several epidemics in the tropics. Most notably, an epidemic in Brazil spanning 2014-2016 linked ZIKV infection to both Guillain Barré syndrome in adults and congenital Zika syndrome in infants. Within the *Flaviviridae* family, insect-specific flaviviruses (ISFVs) such as Aripo virus (ARPV) have been shown to be closely related to pathogenic flaviviruses. We developed an Aripo-Zika (ARPV/ZIKV) chimeric vaccine candidate containing the pre-membrane and envelope genes of ZIKV substituted into the genome of ARPV. Herein, we evaluated the immunogenicity of ARPV/ZIKV using coinfection, passive transfer of antibodies, immunotyping, and epitope mapping data. We performed *in vitro* studies in Vero76s where ARPV and ARPV/ZIKV were each coinfected with Zika. Our results indicate ARPV and ARPV/ZIKV remain incapable of replication in vertebrate cells even during active ZIKV replication. We also conducted a maternal antibody transfer study in immunocompetent mice, where dams were vaccinated and mated with naïve males. ARPV/ZIKV pups (n=4) were completely protected from a ZIKV challenge at 4 weeks old as shown through weight change, survival, and viremic analysis. Also, we used blockade of binding (BOB) assays using ZIKV-specific mAbs to assess epitope-specific ZIKV immunity induced by vaccination. Our results indicate ARPV/ZIKV elicited seroconversion more effectively in immunocompetent mice when compared to our attenuated ZIKV group among multiple distinct epitopes. Lastly, we performed immunotyping of sera from our vaccine studies in immunocompetent mice. Our results indicate ARPV/ZIKV expressed IgG2b, IgG2a, and IgG1 more when compared to our healthy controls. IgA, IgD, IgE, IgM, and IgG3 were expressed at low levels among the control groups and vaccine group. Overall, ARPV/ZIKV continues to be a safe and promising avenue for researching chimeric flavivirus vaccines with evidence of eliciting strong neutralizing antibody responses.

Auranofin Demonstrates Antiviral Effects Against Hepatitis E Virus

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Hepatitis E virus (HEV) is a globally distributed hepatotropic virus that can cause severe disease and death in immunocompromised individuals and pregnant women. While there are currently no approved antiviral therapeutics for treating HEV, Ribavirin and pegylated interferon are currently used off-label and represent the current standard of care for severe HEV infections. Thus, there is an urgent need to discover or repurpose drugs that are safe and efficacious for treating HEV infections. Auranofin, a FDA-approved antirheumatic drug, has been implicated to be repurposed for treating a wide range of ailments, including viral, bacterial, and parasitic infections as well as inflammatory diseases and cancer. Therefore, Auranofin was examined for its antiviral potential against HEV. At non-toxic concentrations, Auranofin displays dose-dependent antiviral activity against HEV in a human hepatocyte cell line using replicon and infectious virus systems. Towards identifying its mechanism of action, Auranofin has been implicated to interact with intracellular redox pathways, specifically enhancing the production of glutathione. Glutathione is deplenished during many different viral infections, including hepatropic infections like HEV. Therefore, this enhancement of glutathione production by Auranofin is being further investigated as a potential antiviral pathway of the drug.

TITLE: A MODEL FOR PREVENTING BAT BORNE RABIES IN COLOMBIA

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Rabies is among one of the oldest and most lethal of all zoonotic diseases. A key reservoir of rabies in wildlife are bats, who have been found to carry the virus across the American continents. In Colombia, the common vampire bat (*Desmodus rotundus)* functions as a main transmissor of the rabies virus to humans, other wildlife, and to domesticated livestock species such as cattle. As such, this species has massive social and economic impacts to the health and agricultural sectors of Colombia. Vaccination has proven to limit the scope and spread of rabies outbreaks, but where and when to target vaccination efforts remains uncertain, especially in resource limited areas. This study aimed to use curated rabies outbreak and agricultural intelligence, targeted field sampling, and country level geographic information to identify vampire bat and rabies outbreak hotspots within Colombia. We also identified key factors which facilitate rabies outbreaks, and used this information to forecast areas in which vaccination can be targeted. Finally, the model developed in this study is expected to inform when and where rabies vaccination should be deployed in hotspots of bat-borne rabies in Colombia. Our modeling framework could be used to elucidate areas of concern for bat borne rabies outbreaks, and for preventative interventions in other countries or regions.

Characterization of Virulence and Metabolic Gene Functions Within Prophage Regions of >200 *Salmonella enterica* Serovars

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Prophage regions within bacterial genomes can carry virulence and metabolic genes in addition to phage related genes. The non-phage related genes that are carried within prophage regions may increase the *in vivo* fitness of the bacterial host. The overall objective of this study was to characterize prophage regions within *Salmonella enterica* subspecies *enterica* (*S. enterica*) isolates to understand their role in the acquisition of novel virulence and metabolic functions in *Salmonella.* We analyzed the whole genome sequence data of 242 *S. enterica* isolates representing 217 different serovars. Phaster annotations for prophage regions were quantified, and the presence/absence of prophage were mapped onto a core SNP phylogeny that was inferred with IQ-TREE and visualized in iTOL. EggNOG was used to classify coding sequences into clusters of orthologous groups (COG) categories to allow for further classification and identification of potential virulence factors and metabolic-associated functions. On average, 3.87% (range: 3.52% - 4.87%) of the genomic content of each isolate was annotated as prophage regions; this was consistent across different phylogenetic clades (p-value = 0.978; ANOVA). Gifsy 1 and Salmon Fels 1 were the two most common intact prophage identified, with none of the nine most common prophage being associated with a specific clade. Among the 37 unique COG categories, we identified virulence factors associated with O antigen conversion, type 1 fimbriae assembly, stress response gene signaling, and siderophore interactions, in addition to gene functions associated with metal ion, sugar, lipid, and anerobic metabolic pathways. Our study provides new information about the cargo that prophages carry, and the role that they may play in the transmission of virulence factors and novel metabolic functions in *Salmonella* that may facilitate expansion of *Salmonella* into a new niche.

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Breakout Session #1: Antimicrobial Countermeasures (vaccine & drug)

Oral Presentations

(in Duckpond)

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Semisynthetic Strategies To Access Novel Chloramphenicol Derivatives

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Chloramphenicol, a peptidyl transferase inhibitor, binds to the 50S ribosome and hinders protein synthesis. Among different pharmacophores this molecule possesses, the *p*-nitro group is uncommon and a known toxicophoric, which may be responsible for the off-target effects of this antibiotic. Indeed, replacing the *p*-nitro group has previously led to better-tolerated chloramphenicol derivatives, such as thiamphenicol. However, this strategy is severely underexplored and relied exclusively on synthetic methods to derivatize the *p*-nitro group rather than the conversion of natural chloramphenicol. Our strategy is directly replacing the *p*-nitro group with different functionalities semisynthetically, creating novel derivatives, and assessing what effect on the activity and toxicity these changes will have. Reduction of the *p*-nitro group led us to the aniline of chloramphenicol, which will be subjected to simple reactions like amidation, diazo coupling, or nucleophilic displacement to create a library of different derivative classes. These derivatives will be investigated further on their activity and toxicity. Top lead molecules will be subjected to additional modifications with a view to further improving the drug.

Engineering an Enhanced Auxin-Inducible Degron Degradation System for Rapid Depletion of Fungal Proteins: Expanding Horizons in Antifungal Drug Target Identification and Protein Function Studies

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The selection of appropriate biomolecular targets is a crucial aspect of biopharmaceutical development. The Auxin-Inducible Degron Degradation (AID) technology has demonstrated remarkable potential in efficiently and rapidly degrading target proteins, thereby enabling the identification and acquisition of novel drug targets. The AID system also offers a viable method to deplete specific proteins, particularly in cases where the degradation pathway has not been exploited, or when adaptation of proteins, including the cell environment, occurs to compensate for the mutation or gene knockout. In this study, we have engineered an improved AID system tailored to deplete proteins of interest. This AID construct combines the auxin-responsive E3 ubiquitin ligase binding domain, AFB2, and the substrate degron, IAA17, fused to the target genes. Essential genes of fungi with the lowest percent amino acid similarity to human and plant orthologs, according to the Basic Local Alignment Search Tool (BLAST), were cloned into the AID construct in S. cerevisiae (AID-tagged strains) using modular yeast cloning toolkit for multipart assembly and direct genetic modification. Each E3 ubiquitin ligase and IAA17 degron was fused to a fluorescence protein, allowing for real-time monitoring of protein levels in response to different auxin doses via cytometry. Our AID system exhibited high sensitivity, with an EC50 value of 0.040 μM (SE = 0.016) for AFB2 ubiquitin ligase binding domain, enabling the specific promotion of IAA17::target protein degradation. Furthermore, we demonstrate how this improved AID system enhances quantitative functional studies of various proteins in fungi. The advancements made in auxin-inducible protein degradation in this study offer a powerful approach to investigate critical target protein viability in fungi, screen protein targets for novel drugs, and regulate intracellular protein abundance, thus revolutionizing the study of protein function underlying a diverse range of biological processes.

Development of novel inhibitors against Venezuelan equine encephalitis virus by targeting capsid-importin interactions

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Abstract:
Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne, positive sense, single-stranded RNA virus that belong to the genus *Alphavirus*. As a zoonotic pathogen, VEEV infects both equines and humans, with associated neurological complications in ~14% of human cases. Due to its low infectious dose, ease of aerosolization and manipulation, this virus is regarded as a potential bioweapon and is classified as a select agent by both the CDC and USDA. However, there are currently no FDA-approved therapeutics or licensed vaccines against VEEV infection in humans. The VEEV capsid protein is an essential virulence factor of VEEV. The capsid protein can simultaneously bind to the host's nuclear import receptors, importin α/β1, and the host export receptor, CRM1 to form a tetrameric complex. This complex accumulates at the nuclear pore channel, halting nucleocytoplasmic trafficking, downregulating host transcription and cellular antiviral response, and ultimately resulting in cell death. Moreover, VEEV TC83 Cm, with a mutated non-functional nuclear localization sequence (NLS) within the capsid, failed to downregulate cellular transcription and antiviral response. This suggests that the nuclear import of VEEV capsid could be exploited as an attractive target for therapeutic development. We hypothesized that small molecule inhibitors capable of disrupting the interaction of capsid with importin α/β1 should increase cellular antiviral response, resulting in reduced viral titers and rescue of cells from VEEV-induced cell death. Hence, we have identified two small molecule inhibitors, **1564** and **I2**, to disrupt capsid:importin α interaction. Computational modeling predicted the target of these compounds to be the NLS-binding site of importin α. Moreover, biochemical assay using AlphaScreen showed that both compounds impacted capsid:importin α interaction with IC_{50} values of 46.3 μM and 74.9 μM for **I2** and **1564**, respectively. Experimentally, these inhibitors were well tolerated by HMC3 microglia cells with CC_{50} of >250 μM and >500 μM for **I2** and **1564**, respectively. The antiviral activity of these compounds was found to be MOI-dependent with ~50% reduction in VEEV TC83 titer recorded at an MOI of 1 and a viral reduction >90% observed at MOIs of 0.1 and 0.01. At MOI 0.1, both compounds resulted in >1 $\log_{10}$ decrease in viral titer with compound **I2** displaying a superior activity. Further, **I2** displayed a better EC_{50} of 2.96 μM, while **1564** an EC_{50} of 5.38 μM against VEEV. Both compounds also rescued infected cells from VEEV-induced cell death. The contribution of the innate immune response in the antiviral effect and rescue of inhibitor-treated cells from cell death will be delineated by monitoring the translocation of antiviral transcription factors as well as the expression of antiviral cytokines and interferon-stimulated genes. Our findings confirm that targeting cellular factor(s) important for viral pathogenesis represent an alternative strategy for novel antivirals development. Future studies will involve further delineating the mechanisms of action of these inhibitors.

Immunogenicity And Replication Capacity Of Recombinant Rotaviruses Expressing Norovirus Proteins

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Norovirus and rotavirus are RNA viruses that cause acute gastroenteritis across the globe. All age groups are susceptible to norovirus whereas rotavirus primarily infects children. Licensed rotavirus vaccines are available, however such vaccines have limitations including lower efficacy rates in low- and middle-income countries and risks of intussusception. No vaccines are available for norovirus. To address this need, we generated candidate, dual norovirus and rotavirus oral vaccines and evaluated their ability to replicate and induce immune responses in gnotobiotic (Gn) pigs. A rotavirus reverse genetics system was used to produce two vaccines. The first vaccine was a recombinant rhesus rotavirus (rRRV) expressing the P domain of norovirus (rRRV-P). In the second vaccine, the P domain of norovirus was expressed and the VP7 of rRRV was swapped for VP7 of the SA11 strain of rotavirus (rRRV/SA11 VP7-P). Gn pigs were divided into four groups and received three oral doses of $5x10^7$ fluorescent focus units of rRRV-P, rRRV/SA11 VP7-P, rRRV, or Diluent #5. Throughout the study, piglets were monitored for virus shedding, diarrhea, and the development of serum antibody responses. At necropsy, blood and intestinal contents were collected for evaluating antibody titers. Antibody secreting cell (ASC) numbers in the ileum, tonsils, facial lymph nodes, spleen, and blood were also quantified. All viruses replicated to high titers in Gn pigs. 33-67% of pigs in all virus-inoculated groups shed virus nasally, with mean peak titers ranging 10^6 to10^{10} genome copies/mL. The rRRV inoculated group had the highest amount of virus in nasal samples, followed by rRRV/SA11 VP7-P and rRRV-P groups respectively. In fecal samples, virus was only detected from pigs inoculated with rRRV or rRRV-P, and the mean peak titer was comparable between groups (1.91×10^8 versus 2.49×10^8 genome copies/mL). Only 4 virus-inoculated pigs developed mild diarrhea that lasted between 0.75-1.25 days on average. At PID2, immunofluorescent staining for the P domain of norovirus and VP6 of rotavirus revealed virus in the nasal cavity, salivary glands, and ileum. Tissues were also stained for rotavirus protein NSP3, which in combination with VP6 indicates active replication. All virus-inoculated animals had detectable levels of VP6 and NSP3 in evaluated tissues. In rRRV-P and rRRV/SA11 VP7-P inoculated animals, colocalization of the P domain and VP6 indicated the rRRV backbone was correctly expressing the foreign norovirus protein *in vivo*. High titers of norovirus specific serum IgG and IgA antibodies were induced by rRRV-P and rRRV/SA11 VP7-P vaccination. Strong rRRV specific IgG and IgA responses were detected in serum, SIC, and LIC by all the virus-inoculated pigs. rRRV/SA11 VP7-P inoculation induced a greater number of norovirus specific IgG ASCs in the blood, ileum, and spleen than rRRV-P inoculation and a greater number of IgA ASCs in all tissues except the ileum and blood. Comparable numbers of rRRV specific IgG and IgA ASCs were detected in all tissues sampled following both vaccinations. Taken altogether, our results show that the rRRV-P and rRRV/SA11 VP7-P vaccines are safe, live oral attenuated vaccines that are ready for viral challenge studies.

Subspecies-Specific Detection Of *Xylella fastidiosa* With CRISPR-Cas12a

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The vascular microbial pathogen *Xylella fastidiosa* (*Xf*) has a high economic impact on agricultural production. Latent infections in asymptomatic perennial fruit and ornamental trees have led to pathogen spread in infected plant material between states, countries, and continents. Different subspecies of *Xf* have different host ranges putting different crops at risk, which calls for the need of subspecies-specific detection methods. CRISPR-Cas12a-based technology is an emerging tool that can provide highly sensitive detection of specific nucleic acid targets, which allows the development of subspecies-specific pathogen detection. In this study, we performed a pangenome analysis using 83 validated *Xf* genomes and identified genes specific to each *Xf* subspecies. All 83 *Xf* genomes (including 49 subspecies *fastidiosa* genomes, 16 subspecies *multiplex* genomes, 12 subspecies *pauca* genomes, three subspecies *sandyi* genomes, two subspecies *morus* genomes, and one *Xylella taiwanensis* genome) were annotated. The protein coding sequences were further analyzed in search of orthologous and unique genes across and within *Xf* subspecies. Based on the subspecies-specific gene sequences, guide RNAs were designed for the detection of subspecies *fastidiosa* and *multiplex*, respectively. Then, we designed primers that amplify the CRISPR (guide RNA) target regions. We successfully established CRISPR-Cas12a detection systems that allow highly specific detection of the targets within 20 minutes. Our results also revealed the potential for integrating microneedle DNA extraction, isothermal DNA amplification, and lateral flow assays with CRISPR-Cas12a systems to construct a rapid easy-to-use detection method for pathogens in plant leaves. Such a detection system is a promising technology for diagnostic biosensor development, although our research uncovered a few limitations (including environmental condition, storage, and handling requirements) and room for improvement in these assays.

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Breakout Session #2: Computational Biology and Disease Modeling

Oral Presentations

(in Smithfield)

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A Synthesis Work on Climate Change and Coastal Ecosystem Health

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Abstract

Linkages between a warming climate and human health have long been recognized, particularly regarding the impact of climatic variation on the prevalence of infectious diseases. In the context of infectious diseases and health, climate change has been generally linked to vector-borne diseases, with its impacts on coastal ecosystems and their link to water-borne pathogens and food security being largely neglected. We analyzed long-term global remote sensing data in coastal areas spanning 1855-present to establish signals of change in sea surface temperature between 1900-present. We then assessed projected sea surface temperature values for 2050, and for 2100, accounting for diverse emission scenarios over the next 75 years. We found steady increases in sea surface temperatures across nearly all regions studied, with a more pronounced increase starting in the 1980's and becoming exacerbated in 2050 and 2100. As a case study we explored relationships between water-borne pathogens and climatic variation in the last three decades in Latin America focusing on *Vibrio*. *Vibrio* are a group of water-borne bacteria of interest to human health who's life cycle and propagation are tied to local thermal constraints and subsequently tied to climate. We used *Vibrio* data from the Global Lancet Countdown project to develop a baseline between 1982 and 1986 of areas suitable for bacteria propagation and cases of vibriosis in each country, and used this model to assess climate-driven variation in *Vibrio* suitability and infections across Latin America. We found that most of Latin America shows a steady increase in conditions suitable for *Vibrio* bacteria in coastal waters since 1982, with countries like Venezuela, Ecuador, and Panama revealing significant association between the increase in area suitable for *Vibrio* and increased cases of vibriosis in humans. Signals of coastal environmental change reveal accelerated sea surface temperature increases in northern regions. In our ongoing study we discuss these findings, and their implications in the context of coastal health, including effects on public health, fisheries, and ecosystem services.

Modeling the Effects of Host Availability and Temperature on Mosquito-borne Parasite Transmission

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Global climate change is predicted to cause range shifts in the mosquito species that transmit pathogens to humans and wildlife. Recent modeling studies have sought to improve our understanding of the relationship between temperature and the transmission potential of mosquito-borne pathogens. However, the role of the vertebrate host population, including the importance of host behavioral defenses on mosquito feeding success, remains poorly understood despite ample empirical evidence of its significance to pathogen transmission. Here, we derived thermal performance curves for mosquito and parasite traits and integrated them into two models of vector-host contact to investigate how vertebrate host traits and behaviors affect two key thermal properties of mosquito-borne parasite transmission: the thermal optimum for transmission and the thermal niche of the parasite population. We parameterized these models for five mosquito-borne parasite transmission systems, leading to two main conclusions. First, vertebrate host availability may induce a shift in the thermal optimum of transmission. When the tolerance of the vertebrate host to biting from mosquitoes is limited, the thermal optimum of transmission may be altered by as much as 5°C. Second, thresholds for sustained transmission depend non-linearly on both vertebrate host availability and temperature. At any temperature, sustained transmission is impossible when vertebrate hosts are extremely abundant because the probability of encountering an infected individual is negligible. But when host biting tolerance is limited, sustained transmission will also not occur at low host population densities. Together, these results suggest that vertebrate host traits and behaviors play essential roles in the thermal properties of mosquito-borne parasite transmission. Increasing our understanding of this relationship should lead us to improved predictions about shifting global patterns of mosquito-borne disease.

ESM_2: A PROMISING DEEP LEARNING APPROACH FOR IDENTIFYING NOVEL ANTIBIOTIC RESISTANCE GENES.

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ABSTRACT:

The discovery of new antibiotic resistance genes is of utmost importance in directing mitigation options for preventing the spread of antibiotic resistance, including informing development of new antibiotics. Currently, a "best-hit" approach through comparing DNA sequences derived from genomic or metagenomic data sets to public databases. However, best hit approaches often yield false negatives and can fail to recognize new gene mutations, particularly when they are highly dissimilar from sequences available in public databases. To overcome such limitations, here we introduce the ESM-2 deep learning algorithm, a deep learning model designed to understand and differentiate sequences within the training dataset..

Within this study, we explore the performance of two distinct methodologies —for detecting novel antibiotic resistance genes (ARGs) using the HMD-ARG dataset BLAST (employing the "best-hit" approach) and ESM_2. HMD-ARG dataset is extensivley manually currated database consiting of 17,282 sequences with annotated labels for 15 antibiotic classes, 6 resistance mechanisms, and mobility information. The dataset was partitioned using CD-HIT with a 40% similarity threshold, allocating 80% to the training dataset and the remaining 20% to the test dataset. Notably, the maximum accuracy achieved by BLAST was only 23.06%. Notably, accurate ARG detection was confined to the tetracycline, glycopeptide, and macrolide-lincosamide-streptogramin (MLS) classes within the training dataset.

ESM_2 achieved a threefold enhancement in overall accuracy on the test dataset compared to BLAST. This advantage spanned across ARGs spanning all 15 antibiotic classes in the HMD-ARG dataset, showcasing heightened precision, F-1 scores, recall, and accuracy.

These findings establish ESM_2 as a promising candidate for identifying novel ARGs. However, this potential breakthrough comes with an important consideration of ESM_2's demanding computational prerequisites. Operating with 8 million parameters, the model requires several gigabytes of memory to function effectively.

Keywords: Antibiotic Resistance Genes, BLAST, ESM_2, CD-HIT 40, Deep Learning, HMD-ARG dataset.

Title: Ecological Niche Models of Cache Valley Virus; An Emerging Orthobunyavirus in North America

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Abstract:

Cache Valley Virus (CVV) is an understudied *Orthobunyavirus* with high spillover potential due to its wide geographic range and large number of various potential hosts and vectors. Although CVV is known to be widespread throughout North America no studies to date have attempted to quantify its true distribution. We used ecological niche models to estimate both the geographic range of CVV, as well as correlate its range with potential vectors and hosts to estimate which species are the most likely sylvatic contributors. We found that CVV is widespread throughout North America and likely has multiple primary vector species including *Aedes vexans*, *Culiseta inornata*, and *Culex tarsalis* that shift across the virus's range, while its primary host is *Odocoileus virginianus* (White-tailed Deer). We also found large areas of North America with high risk of CVV transmission but had no confirmed CVV reports. We believe this is likely due to misdiagnosis or underreporting which necessitates increased surveillance.

MATHEMATICAL FORMULATIONS FOR REPRESENTING HUMAN RISK RESPONSE IN EPIDEMIC MODELS

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Abstract:
Many models have been created to understand the spread of COVID-19 throughout different populations. Understanding its spread will lead to better preparedness for subsequent outbreaks of COVID-19 or another infectious disease of a similar magnitude. However, COVID-19 has a variety of complexities impacting its trajectory; as a result, many models have poor predictive abilities for estimating caseloads and deaths. One major component initially missing from these models was an effective method for considering the impact of human behavior on disease transmission. A variety of models emerged which explicitly built human behavior into disease models, often coupling models for human behavior dynamics and disease dynamics. We consider various methods that account for human behavior when modeling outbreak behavior, exploring the hypothesis that, when modeling human behavior dynamics, choosing a different approach changes mathematical results. Methods range from varying the (traditionally constant) transmission parameter with respect to time to creating a network model coupling an SIR-type model with a social dynamics network. Of particular interest are models which consider *risk response*, where humans change their actions in response to their perception of likelihood of infection (or death due to infection). Examples of these behaviors include social distancing, isolation, or masking. This mechanism is incorporated into the model so that human response to new information (risk responsiveness) affects disease transmission. Models represent this effect either exogenously or endogenously. With the former incorporation, the behavioral changes are formulated independent of the epidemiological model. With the latter incorporation, behavior shifts are dependent on the changes in the epidemiological model. Risk response allows for the presence of oscillations in model solutions, representing the outbreak waves observed within the pandemic.

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Breakout Session #3: Ecology, Epidemiology, & Environmental Microbiology

Oral Presentations

(in Cascades)

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Title

Trait-based modelling of predator-prey dynamics in vampire bat rabies endemic countries

Authors

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Affiliations

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Abstract

The common vampire bat (*Desmodus rotundus*) is a flying mammal of sanguivorous diet that preys on a variety of taxa to meet its complex nutritional needs. The sanguivorous feeding behavior of vampire bats causes the spread of rabies virus to their prey species. Vampire bat rabies spillover transmission is a widespread but highly complex phenomenon occurring only in tropical Latin American. Studies on the identification of wildlife and domestic species depredated by the common vampire bat are scarce, which hinders the early detection of vampire bat-borne rabies spillover events in the sylvatic and domestic cycles. Identifying and understanding the ecological and functional traits of prey species is expected to have a positive effect on prey selection. A comprehensive list of prey species of the common vampire bat can provide important insights into prey community structure, feeding behavior, and more accurate anticipation of rabies spillover events in novel species. This study aimed to conduct predictions of prey species of the common vampire bat in Latin America using machine learning profiling. We collected x functional and ecological traits for 1936 terrestrial mammalian species in Latin America data from AnimalTraits, AnAge, COMBINE, EltonTraits 1.0, Phylocine 1.2.1, Life History Characteristics of Placental Nonvolant Mammals, and PanTHERIA databases. We compared each trait among different databases using regression modeling to select the most suitable database and to fill data gaps for some species. We found that our method to combine databases for specific traits increased species records and reduce the missing information. Our pilot project employs classification trees and hypervolumes to identify the trait combination most likely representing prey species for common vampire bats. Using calibration and evaluation datasets we were able to develop accurate reconstructions of known prey (n=120) and forecasted new, potential prey species. Our trait-based prey identification helped identify the geographic areas and species to prioritize active epidemiological surveillance of rabies to inform precision-epidemiology efforts to control and prevent rabies spillover at the wildlife-human interface in Latin America.

Key words: Common vampire bat, Latin America, Predator-prey interaction, Rabies, Trait-based modeling

DISTRIBUTION OF THE BATS OF MALI

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Abstract

Bats are considered natural reservoirs for the Ebola virus and other viruses in the West African region where outbreaks of Ebola have occurred. Mali and other West African countries are under public health alert regarding bat-borne pathogens linked to zoonotic diseases. There is, however, a gap of knowledge about the distribution of bats in Mali. To fill this gap, we developed comprehensive ecological niche modelling assessments of the likely geographic distribution of 38 bat species reported for Mali. We used MERRAclim for our predictor variables, and Mobility-oriented parity analysis for assessing strict extrapolation areas in our predictions. Ecological niche models revealed that most suitable areas for the bat species clustered in southern Mali, with few species had suitability areas in the northern region of the country, where arid landscape are dominant. This contribution provides the first detailed estimate of the potential distribution of bats in Mali. The analytical protocol employed can be used for different taxa and different regions. Results provide valuable information to guide wildlife conservation and management, guide research, and inform epidemiological surveillance of bat-borne pathogen transmission to address public health concerns.

ANCIENT GENE TRANSFERS AND CO-EVOLUTION BETWEEN EUKARYOTES AND GIANT VIRUSES

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Members of the phylum *Nucleocytoviricota*, also called "giant viruses" due to their large physical dimensions and genome lengths, are a diverse group of dsDNA viruses that infect a wide range of eukaryotic hosts. Nucleocytoviruses likely evolved from smaller viruses, but the timing of their emergence and its relationship to the early evolution of eukaryotes remains unclear. Recent work has shown that the genomes of nucleocytoviruses often encode Eukaryotic Signature Proteins (ESPs) - including histones, vesicular trafficking factors, cytoskeletal components, and elements of RNA and DNA processing - that occur only rarely outside of eukaryotes. To investigate patterns of gene exchange between viruses and eukaryotes and possibly shed light on the early evolution of both, we examined the occurrence of viral-encoded ESPs (vESPs) and performed a comprehensive phylogenetic reconstruction on a subset that are widespread in nucleocytoviruses. Our results demonstrate that vESPs involved in cytoskeletal structure, ubiquitin system, and vesicular trafficking were acquired multiple times independently by nucleocytoviruses at different timepoints after the emergence of the eukaryotic supergroups. In contrast, vESPs involved in DNA and RNA processing are placed deep in their respective phylogenies, indicative of ancient gene exchange between nucleocytoviruses and eukaryotes. Examination of vESPs that could be rooted in archaea revealed that nucleocytoviruses likely acquired some of these genes prior to the emergence of the last eukaryotic common ancestor (LECA). Importantly, our findings also suggest reciprocal gene transfer from eukaryotes to giant viruses and back to the eukaryotes underscoring the importance of viruses for eukaryotic evolution. Collectively, these results suggest that gene exchange between nucleocytoviruses and eukaryotes played important roles in the evolution of both prior to the emergence of LECA.

Keywords: giant viruses, *Nucleocytovoricota*, eukaryotic signature proteins, viral origins, viral diversity

THE IMPACT OF BREWERY BYPRODUCTS AS A FEED ADDITIVE ON FARMED RAINBOW TROUT (*ONCORHYNCHUS MYKISS)*: SPENT YEAST (*SACCHAROMYCES CEREVISIAE)* AND BLACK SOLDIER FLY (*HERMETIA ILLUCENS)* CULTURED ON SPENT GRAIN

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The brewery byproducts, brewer's spent yeast (BSY, *Saccharomyces cerevisiae*) and brewer's spent grain (BSG), currently have little-to-no value. However, they often contain valuable properties and have the potential to be utilized as a beneficial feed additive by the aquaculture industry. This study examined the ability of hydrolyzed, BSY to be used as a feed additive in rainbow trout (*Oncorhynchus mykiss)* diets to potentially improve production and health in relation to their gastrointestinal bacterial communities. Moreover, this study also examined black soldier fly (BSF) as a feed additive. BSF was cultured on BSG, to convert a high fiber waste product into a product that is nutritious in both quality proteins and fats for fish.

These innovative products were used as a feed additive in rainbow trout (*Oncorhynchus mykiss)* diets as a prebiotic. For both studies, fourteen juvenile rainbow trout (28.6 ± 0.4 g, mean ± standard error) were distributed into 21 polyethylene tanks (170 L each) in a single recirculating aquaculture system (RAS). For the BSY study, three treatment groups were performed in quadruplicate at the tank level, included a control (commercial feed), Low Yeast (LY, commercial feed coated with 2% BSY), and High Yeast (HY, commercial feed coated with 5% BSY). For the BSF study, three treatment groups, performed in triplicate at the tank level, included a control (commercial feed) and the commercial feed individually coated with 3% of three different BSF products: BSF1, BSF2, and BSF3.

In the BSY study, compared to the control, the LY and HY fed fish grew significantly ($P < 0.01$) faster with percent differences of 20.8 and 35.4%, respectively. Between treatment groups, no statistical significance was observed for fish biometrics. Within the adherent mucus, 16S rRNA analysis determined that the bacterial communities exhibited trends of increasing diversity correlated with concentrations of yeast coating. Moreover, significant differences ($P < 0.05$) in the bacterial communities were observed between whole intestinal homogenates and adherent mucus as determined via PERMANOVA and confirmed through Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC). With regards to BSF, the BSF1, BSF2, and BSF3 fed fish also grew significantly ($P < 0.01$) faster than the control fish with percent differences of 34.9, 24.9, 28.8%, respectively. Biometric analysis yielded no significant differences, but 16S rRNA analysis revealed a trend in increasing diversity within trout bacterial communities between each fed BSF diet. Moreover, PERMANOVA and following ANCOM-BC determined significant differential abundances in organisms with very high levels from the *Firmicute* phylum in BSF1 and BSF3.

Feed supplemented with BSY served as a growth promoter for trout, while supporting good animal health. Meanwhile, supplementing feed with BSF, not only served as a growth additive, but also positively shifted the bacterial communities of phylum known to contain prebiotic-influenced organisms. This demonstrates that both BSY and BSG fed BSF can act as beneficial supplements to the diets of rainbow trout by promoting growth, while maintaining good animal health.

Entomopoxvirus Associated Polinton-like Viruses Provide Insight into Replicon Evolution

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Viral satellites are genetic hyperparasites, parasites of other parasites, that rely on host or 'helper' viruses for their own propagation and spread. Viral satellites are found throughout the tree of life, being present in each domain. Historically, recognized eukaryotic satellites have been small entities, with RNA or single stranded DNA genomes less than 6kb, and few to no coding sequences. This trend has been challenged in recent years with the discovery polinton-like viruses (PLVs). PLVs are dsDNA capsid-encoding elements that integrate into eukaryotic cell chromosomes, and have genomes in the range of ~15-25kb. Here we have analyzed the genomes of three novel PLVs isolated from entomopoxvirus derived occlusion bodies formed during infection of two lepidopteran host species. The association of these PLVs with entomopoxvirus occlusion bodies provides compelling evidence for these elements possessing a satellite lifestyle and is the first suggestion of a PLV parasitizing poxviruses. Aligned with this hypothesis, we observe evidence of horizontal gene transfer between these PLVs and entomopoxviruses. Among these entomopoxvirus associated PLVs, we see two lineages of structural gene modules. These lineages associate with divergent replication modules and possess capsid genes highly diverged from other PLV lineages, demonstrating previously unrecognized diversity amongst eukaryotic small dsDNA viruses. We also observe replicon gene turnover amongst related elements and propose a mechanism to explain these patterns.

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Breakout Session #4: Human Dimension of Infectious Diseases, Public Health, and Clinical Microbiology

Oral Presentations

(in Duckpond)

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TITLE: The Knowledge, Practices and Attitudes associated with Malaria Endemicity in the Tribal Communities of Southeastern Bangladesh

ABSTRACT

While malaria is close to elimination in Bangladesh, the disease is still endemic in 13 out of 64 districts, with over 90% of cases reported in the Chittagong Hill Tract (CHT) districts of Bandarban, Khagrachhari, and Rangamati. These districts have hilly topography and are at a higher elevation than the districts free from malaria, which is contradictory to the usual topographic setting of the disease. Most districts within Bangladesh have been malaria free for many years despite having optimum topographic and ecological conditions for the disease while the CHT districts continue to be burdened with malaria. The study aims to investigate the knowledge, attitudes, and practices (KAP) that might allow malaria to persist in the endemic area of Bangladesh. Data was collected using a mixed method approach within the Lama and Alikodom subdistricts of Bandarban district, by performing KAP surveys and key informant interviews and collecting household location data of malaria cases. The locations identified to have the highest concentration of cases are tribal villages within the district. The results suggest that residents of these tribal villages are lacking basic knowledge related to malaria and malaria prevention. They were unaware of how the disease came to be and how it was affecting them. Their perception of effective malaria prevention practices does not coincide with established recommendations by public health officials. A close relationship between their occupational choices and malaria afflictions was found. Our findings can inform policy as Bangladesh continues to move toward eliminating malaria from within the nation's borders saving the tribal minority from the burden of malaria.

MORTALITY IN MEXICO CITY DURING THE 1890 INFLUENZA EPIDEMIC

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The “Russian flu” was a respiratory illness of unknown cause which sparked a global pandemic between the years 1889 and 1892. As the disease spread, health organizations and the public sought information about the number of cases and deaths. Given the limited data available at the time, the public formed its opinions on the pandemic through information gleaned from reports and anecdotes presented in the press, a trend that continues to the modern day, albeit in a digital fashion. This research focuses on mortality in Mexico city during the first few months of the influenza outbreak in 1890 and seeks to answer questions related to the severity of the disease, the number of fatalities, and historical interpretations of these figures. Using keyword searches in a database of historical Mexican newspapers and translating relevant articles and statistical tables, data was collected on daily, weekly, and monthly mortality totals. The data was analyzed by cause of death and compared to previous years to measure excess mortality. A close reading of these primary historical sources gave insight into how understandings and attitudes changed in the face of conflicting information. They revealed controversies about data omission in the press, the harsh implications of social class during a public health emergency, frustrations with the inaction of authorities, and personal stories of fear and grief. The analysis of this research draws parallels to other respiratory pandemics – most recently, COVID-19 – through its discussion of how an epidemic takes shape in statistical measures as well as public perceptions. This project seeks to further our understanding of the complexities of societal responses to infectious disease outbreaks.

Understanding Impacts from Inflow and Infiltration on Pathogen Detection and Levels in a Rural Sewershed: Toward Universal Application of Wastewater Based Surveillance and Epidemiology

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Despite the advantages of wastewater-based surveillance (WBS) for monitoring community health, it has largely shown the most promise in densely populated communities with well-maintained and funded sewer infrastructure. Consequently, there is a lack of knowledge base on how rural communities in the U.S. can take advantage of WBS to understand existing health disparities and subsequently design more targeted public health interventions. In this study, we conducted a year-long monthly wastewater monitoring campaign in a small, rural sewershed in Southwest Virginia with known infrastructural challenges, including sewer main leaks and groundwater and surface water infiltration and inflow (I&I). In addition to collecting wastewater treatment plant (WWTP) influent, we sampled 12 sewershed nodes at each branch line to the sewer conveyance system to capture pathogen signal loss due to I&I and sewer system design characteristics. TaqMan Array Card (TAC) qPCR was performed for simultaneous quantification of 54 enteric pathogen targets. After twelve months of data collection, *Giardia lamblia*, Norovirus GII, and Adenovirus were present in 86%, 11%, and 32% of WWTP influent samples respectively. Due to a 22% lower detection rate of pathogen signal at the influent as compared to downstream of a large residential facility, preliminary results point to underestimation of pathogen circulation when sampling influent alone in a community with persistent I&I, informing design considerations for WBS implementation in rural areas.

Inhibition Of Stress Hormone Receptors Reduces Clinical Recurrences Of Herpes Simplex Virus 1 In Guinea Pigs

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Herpes simplex viruses 1 and 2 (HSV1 and HSV2) establish lifelong latency in sympathetic and sensory neurons. In both humans and animal models, stress is known to cause reactivation of HSV leading to subsequent clinical symptoms such as oral and genital lesions. The stress hormone epinephrine mediates the "fight or flight" response by binding to adrenergic receptors (AR) found on numerous cell types throughout the body, including sympathetic and sensory neurons. We previously showed *in vitro* that epinephrine causes reactivation of HSV1 in sympathetic neurons, but not sensory neurons, and reactivation requires the activation of multiple adrenergic receptors, specifically α2-, β1-, and β2-ARs. We hypothesized that a combination of α2-AR and non-specific β-AR antagonists would decrease the frequency of clinical recurrences of HSV1 *in vivo*. Hartley guinea pigs were intravaginally infected with HSV1. After acute infection (14 days post infection), guinea pigs were either untreated or treated with Atipamezole (α2-AR antagonist), Propranolol (non-selective β-AR antagonist), or a combination of Atipamezole/Propranolol. Each guinea pig was weighed, treated with an AR antagonist, vaginally swabbed, and examined for genital lesions daily. Treatment with Atipamezole alone reduced recurrences, but recurrences were further reduced by treatment with both Atipamezole and Propranolol or Propranolol alone. Clinical data suggests that adrenergic antagonists, particularly beta blockers, may be useful for reducing the frequency of HSV1 recurrences. Daily vaginal swabs are currently being processed by plaque assay and qPCR to determine if blocking ARs may also have an impact on asymptomatic viral shedding in vaginal secretions.

SARS-CoV-2 Omicron XBB1.5 Shows Altered Replication In Neurons Versus Ancestral WA1/2020, Facilitated By NRP-1

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Despite official announcements of the COVID-19 pandemic's conclusion, the global spread of SARS-CoV-2 persists. A significant portion, approximately 80%, of individuals display neurological symptoms during the acute phase of COVID-19, with around 85% of Long-COVID patients reporting similar symptoms during the post-acute phase. Our prior research has demonstrated that SARS-CoV-2 rapidly infiltrates the peripheral nervous system via direct invasion prior to viremia, leading to productive infections and sensory impairments. Additionally, we've revealed that the virus enters neurons through interactions with the host protein neuropilin-1. These studies were conducted using the ancestral SARS-CoV-2 strain (WA1/2020), predating the emergence of subsequent variants including alpha, beta, gamma, delta, and omicron. To understand how contemporary SARS-CoV-2 variants compare in terms of replication kinetics to WA1/2020 we infected primary neuronal cultures from k18-hACE2 mice and wild-type (WT) mice with the Omicron variant XBB1.5. We collected neurons and media separately up to five days after infection. Ganglia assessed included the trigeminal ganglia (TG), responsible for sensory innervation in the head, including the nasal septum; the superior cervical ganglia (SCG), which supplies sympathetic innervation to glands and vessels in the head; and the dorsal root ganglia (DRG), which transmits sensory signals from organs/periphery to the spinal cord. These ganglia have been linked to neurological disorders reported by Long-COVID patients: TG (trigeminal neuralgia), SCG (Horner syndrome), and LS-DRG (radicular pain). Viral genome replication and release were assessed in neurons and media, respectively, using RT-qPCR, while infectious virus production and release were quantified using plaque assays. RT-qPCR showed that similar to WA1/2020, XBB1.5 was capable of entering neurons and replicating its genome across different ganglia and mouse types. Interestingly, XBB1.5 replicated its genome to higher RNA concentrations (>1 log) in WT neurons compared to WA1/2020, while peak RNA concentrations were similar between the strains in hACE2 neurons. This increase could be due to heightened utilization of neuropilin-1 as an entry factor. Plaque assays confirmed the release of infectious virus from DRGs in both WT and hACE2 neurons one day post infection, in contrast to WA1/2020. To investigate the involvement of NRP-1 in the entry of both strains, a fluorescently labeled translation blocking antisense morpholino oligonucleotide (MO) was used to knockdown NRP-1 expression in primary neuronal cultures of DRGs prior to infecting them with either WA1/2020 or XBB1.5. Neurons and media were collected together two days after infection, corresponding to the initial surge in genome replication as previously established; viral RNA concentrations were then assessed using RT-qPCR. Viral RNA concentrations were significantly reduced for both viruses in the MO treated cultures compared

to control cultures. In conclusion, our study shows that XBB1.5 exhibits increased replication kinetics compared to the ancestral strain, especially in WT neurons; neuronal infection is productive, with transient release of infectious virus in DRGs; and that neuropilin-1 is a crucial entry factor for both WA1/2022 and XBB1.5. Our findings offer valuable insights into the neuroinvasive behavior of contemporary SARS-CoV-2 variants, paving the way for further investigation of functional consequences of these differences in future in vivo studies.

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Breakout Session #5: Immunology and Host-Pathogen Interactions

Oral Presentations

(in Smithfield)

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Rift Valley fever virus NSs protein interacts with LC3 family members to inhibit antiviral autophagy.

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Rift Valley fever virus (RVFV) is a negative-sense RNA arbovirus (*Phlebovirus* genus, *Phenuiviridae* family, *Bunyavirales order*), endemic in sub-Saharan Africa, that infects both ruminants and humans. Transmission occurs via mosquitos, contact with blood or amniotic fluid of an infected animal, vertically from mother to offspring, or via virus-laden aerosols. Studies identified several competent RVFV vectors such as *Cx. pipiens* mosquitoes in the US and Europe. Impregnated ruminant infections are characterized by abortion storms and fetal malformations in which spontaneous abortion occurs in approximately 100% of cases. Ruminant disease is severe with mortality rates up to 100% in young ruminants and 30% in adults, perilously causing severe socio-economic impacts. In humans, the infection is typically mild, with symptoms including headache, muscle pain, and fatigue. Ten percent of cases progress to a severe version of the disease that includes hemorrhagic fever or encephalitis. Despite its pathogenic potential and economic impact, there are no FDA-approved therapeutics or vaccines to challenge the global spread of this infectious organism. The nonstructural small (NSs) protein is the main virulence factor of RVFV, making it an attractive antiviral target. Bioinformatic and structural analysis identified four potential LC3-interacting region (LIR) motifs in the RVFV NSs protein, suggesting that NSs forms polyvalent interactions with LC3, the host key autophagy protein. Autophagy is a homeostatic process in which cellular materials are degraded and can be either proviral or antiviral. To determine whether NSs interacts with LC3-family proteins, isothermal titration calorimetry (ITC) experiments were performed with peptides corresponding to the predicted LIRs. ITC demonstrated that LIR4 interacts with high affinity with all six LC3 proteins, whereas weak or no binding was observed with LIR1-3. To confirm the NSs-LC3 interaction, plasmids encoding LC3-family members were utilized, and co-immunoprecipitation confirmed that NSs interacts with all six LC3-family members in RVFV-infected cells. NSs also co-immunoprecipitated with endogenous LC3A and LC3B in RVFV-infected cells. Substitution of key amino acids in LIR4 of NSs resulted in significant loss of binding to LC3B in infected cells, indicating a crucial role for LIR4. Nuclear-cytosolic trafficking of LC3 regulates autophagy initiation thus, experiments were performed to determine location of the NSs-LC3 interaction. Cellular fractionation followed by co-immunoprecipitation demonstrated the NSs-LC3 interaction occurred predominantly in the nucleus. Confocal microscopy demonstrated that NSs colocalized with LC3A in perinuclear and filamentous regions, suggesting NSs is

sequestering LC3A in the nucleus to prevent antiviral autophagy. This is supported by experiments demonstrating that NSs downregulates autophagy through LIR4. These results demonstrate that RVFV NSs inhibits antiviral autophagy through interaction with LC3-family proteins, providing another mechanism that RVFV NSs dampens the host antiviral response. There is a high likelihood that RVFV will emerge in new locations or cause significant outbreaks in current endemic areas. Thus, our research to understand the viral NSs-host LC3 interaction as a future therapeutic target is of critical importance to public health.

FUNCTIONAL CHARACTERIZATION OF A NOVEL KINASE TgTKL1 IN *TOXOPLASMA* PATHOGENESIS

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Abstract

Toxoplasma gondii, the causative agent of toxoplasmosis, is an important pathogen that affects nearly one-third of the human population worldwide. *Toxoplasma* has many clinical forms ranging from asymptomatic to lethal depending on the host immunity and the stage of infection. In pregnant women, infection causes miscarriage, while in newborn children, blindness and cognitive impairments can occur. More importantly, in immunocompromised individuals, *Toxoplasma* infection will result in fatal encephalitis. Current available treatments are compromised by toxic side effects and ineffectiveness against chronic form of the parasite, which urges the need to develop new therapeutic strategies. Therefore, identifying novel and unique parasite factors important for pathogenesis becomes a priority to combat toxoplasmosis. Tyrosine Kinase-Like (TKL) family proteins are plant like kinases that are predicted to play critical roles in *Toxoplasma* growth, but are poorly studied. We focused on the nuclear kinase TgTKL1, and our studies showed that this kinase is important for *Toxoplasma* growth *in vitro* and essential for virulence *in vivo*. TgTKL1 contains four different domains: RNI domain, Enhanced Disease Resistance 1 (EDR1) domain, kinase domain and Nuclear Localization Signal (NLS) motif and contributions of these domains to TgTKL1 function remain unknown. To define the role of these domains in TgTKL1 function, we generated different domain mutant strains using CRISPR-Cas9 based editing platform. To determine the significance of kinase domain, we successfully generated a kinase mutant strain and interestingly, it displayed defects in growth and virulence like TgTKL1 null mutant. RNA-seq analysis showed that invasion related genes are downregulated in the kinase mutant including TgSUB1 (subtilisin 1), a protease required for microneme proteins processing during host-cell invasion. Accordingly, TgTKL1 kinase mutant displayed impaired processing of micronemal proteins revealing that kinase activity is crucial for TgTKL1 function. Furthermore,

we generated the NLS mutant and interestingly this mutation results in mis-localization of TgTKL1 to the cytoplasm. Additionally, transcriptomics analysis revealed that gene expression profile of the NLS mutant is quite similar to the kinase mutant. Moreover, TgTKL1 NLS mutant showed impairment in the processing of micronemal proteins, revealing that nuclear localization is also essential for TgTKL1 function. Currently, we are in the process of generating RNI and EDR1 deletion mutants and once these strains are generated, they will be subjected to different phenotypic assays including growth, invasion, microneme secretion and virulence. Furthermore, multiple approaches including quantitative phosphoproteomics, immunoprecipitation and proximity ligation assays will be used to understand the signaling pathways mediated by TgTKL1.

MDM2 Overexpression Reduces Venezuelan Equine Encephalitis Virus Production Independent of its E3 Ligase Activity and the Proteasome

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Venezuelan equine encephalitis virus (VEEV) is a mosquito transmitted virus which can cause long-term neurological deficits in both humans, equines, and laboratory animals. It poses significant risk to the general population due to its potential for use as a bioweapon, due to ease of aerosolization, and lack of publicly available treatment options; therefore, it is of great importance that we develop therapeutics against the long-term neurological sequelae induced by VEEV. The Ubiquitin-protease system has previously been identified as an important antiviral factor across several virus families including the new world and old-world alphaviruses. Here we investigate one component of the ubiquitin-protease system, the E3 ligase mouse double minute 2 (MDM2) for its antiviral potential against VEEV. Given that VEEV capsid can be ubiquitinated, a potential interaction between MDM2 and capsid was evaluated. Co-immunoprecipitation and confocal microscopy showed an interaction and co-localization between the VEEV capsid protein and MDM2. MDM2 overexpression significantly inhibited VEEV infectious titers with a corresponding decrease in VEEV capsid protein expression at 4,8, and 16 hours post infection. Conversely, overexpression of MDM2 had no impact on VEEV TC-83 Cm, which encodes capsid with a mutation in its nuclear localization sequence. Overexpression of MDM2 RING domain mutant C464A, which lacks E3 ligase activity, also significantly reduced VEEV infectious titers. In addition, treatment of cells with the proteasome inhibitor MG-132 was unable to rescue VEEV titers from MDM2 overexpression. These data indicate that MDM2's ability to inhibit VEEV replication is independent of its E3 ubiquitin ligase activity and the proteasomal degradation pathway. Given that previous studies have shown the MDM2 can bind to cellular mRNA and modulate translation, ongoing studies are targeted at determining the impact of MDM2 on viral translation.

Induction of an Endogenous Giant Virus in *Chlamydomonas reinhardtii*: Friend or Foe?

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Nucleocytoplasmic large DNA viruses (NCLDV), commonly referred to as "giant viruses", often encode for complex genomic repertoires that may have impacted the evolution of protist lineages. Recent studies have shown that the genomes of giant viruses can endogenize into the genomes of a broad range of eukaryotic algae, changing their content to varying degrees. *Chlamydomonas reinhardtii* is a model unicellular green alga that has been studied for decades, but for which no virus has been described to date. We analyzed high-throughput genomic sequencing data of thirty-three *C. reinhardtii* field isolates and found six isolates that harbor near-complete signatures of Giant Endogenous Viral Elements (GEVEs) from two distinct lineages of giant viruses. Among eukaryotes, the only other well-documented case of DNA viruses integrating their genomes into those of their hosts is observed in viruses that infect brown algae. Considering the new insights gained on GEVEs in recent years, it is important to redirect efforts to better understand the infection cycles of these latent and novel eukaryotic algal viruses. Using a multifaceted approach that incorporates molecular identification, flow cytometry, transcriptomics, and electron microscopy, we have been able to detect viral induction in one strain of *C. reinhardtii* harboring a GEVE. In addition, we used Oxford nanopore long-read sequencing to identify the GEVE insertion site within the *C. reinhardtii* genome. This is the first evidence of *C. reinhardtii* interacting with giant viruses in nature, which could help develop a potential NCLDV-host model system to study these interactions and understand the consequence of giant virus endogenization on eukaryotic genome evolution.

Cross Protection Against Usutu Virus And Saint Louis Encephalitis Virus In Mice Treated With West Nile Virus Convalescent Plasma

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Usutu virus (USUV), Saint Louis encephalitis virus (SLEV) and West Nile (WNV) virus are mosquito-borne flaviviruses. They are positive-sense single-stranded RNA viruses and are within the Japanese encephalitis serocomplex. They are highly virulent to birds and can cause damage to their lungs, spleens, livers, kidneys, & brains. In humans, it sometimes remains asymptomatic but can cause fever, skin rash and damage in the central nervous system that results in neuroinvasive diseases such as meningitis, encephalitis, and meningoencephalitis. These viruses follow a similar transmission cycle. Their hosts are passerine birds and vectors are *Culex* sp. mosquitoes. Currently, USUV and WNV are co-circulating in many European countries, and SLEV and WNV are co-circulating in the United States. Thus, it is possible that an individual could get infected sequentially with these viruses. Therefore, previously we investigated if West Nile virus vaccination protects against USUV infection in mice lacking a functional type I interferon receptor (*Ifnar*-/- mice). We observed significant protection from USUV disease in WNV vaccinated mice compared to mock vaccinated mice. Therefore, to study whether USUV is cross-neutralized by WNV induced antibodies, we tested cross-neutralization of USUV by human WNV convalescent plasma *in vitro*. At first, we confirmed that 5 of our plasma samples neutralized WNV. Then we tested cross-neutralization of USUV, SLEV, and JEV. We found WNV-positive samples cross-neutralized the tested viruses USUV, SLEV, and JEV. In the future, we will conduct an *in vivo* study by treating wild type mice (C57BL/6) with WNV-specific human convalescent plasma followed by challenging them with USUV and SLEV. Disease conditions will be observed by measuring their weights, viremia in blood, and observing histopathology, especially in the brain. This study will reveal if anti-WNV antibodies cross-neutralize USUV and SLEV in mouse models. This study will help develop vaccines against WNV, SLEV, and USUV and predict disease consequences for individuals exposed to multiple flaviviruses.

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Oral Presentations

(in Cascades)

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Chromosomal Inversions Differentiate Mosquitoes in *Culex Pipiens* Complex

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Chromosomal inversions are of fundamental importance for the process of evolution and are associated with critical traits related to the epidemiology in malaria mosquitoes from genus *Anopheles.* Mosquitoes from the *Culex pipiens* complex serve as primary carriers of encephalitis viruses, including the West Nile virus, which is linked to the prevalent arboviral illness in the United States. Unlike other mosquitoes, species from this complex are highly opportunistic in their choice of hosts, which includes humans, mammals, and birds. Other unique features of *Culex* mosquitoes include their abilities to produce first round of eggs without a blood meal and to use both polluted and non-polluted water reservoirs as a larval habitat. Resistance of *Cx. pipiens* and *Cx. quinquefasciatus* to various insecticides is widely spread in natural populations. However, it remains unknown if any of these important phenotypic features and genetic diversities are associated with chromosomal inversions in *Culex* mosquitoes. Inversions have never been characterized in mosquitoes from genus *Culex* due to the poor quality of their polytene chromosome. In this study, we utilized a Hi-C proximity ligation approach, which belongs to the molecular-based 3C (Chromosome, Conformation, Capture) methods, and provides insights into 3D-organization of the genomes. We employed the Hi-C methodology on three strains of *Culex pipiens pipiens*, two from North America and one from Eurasia, as well as one strain of each *Cx. p. molestus* from North America and *Cx. quinquefasciatus* from Africa. Our analysis revealed a total of eight chromosomal inversions, ranging in size from 2 to 15 megabase pairs. Inversions were unevenly distributed between chromosomal arms with most of them located in arms 1p and 3q. Although all chromosomal inversions were polymorphic, they were taxa specific among three examined members of *Cx. pipiens* complex. Our findings suggest that inversions are highly abundant in natural populations of mosquitoes from the *Cx. pipiens* complex and can potentially contribute to ecological adaptations and taxa diversification in this group of mosquitoes.

The Role of *Culex territans* Mosquitoes in the Transmission of *Batrachochytrium dendrobatidis* to Amphibian Hosts

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Amphibian populations are in decline worldwide. The chytrid fungus, *Batrachochytrium dendrobatidis (Bd),* is a major contributor to this decline, causing mass die offs and extinctions in amphibians. The fungus infects the skin of amphibians, causing deadly chytridiomycosis. This pathogen is thought to be spread primarily through direct contact between frogs, though vector transmission has not been studied. *Culex territans* is a mosquito that is known to feed on amphibians and reptiles. *We hypothesize* that, since *Cx. territans* is in direct contact with the skin of frogs during feeding, this mosquito can vector *Bd*. Transmitting this pathogen indicates that this mosquito could contribute to decline in amphibian health. We tested this hypothesis by screening blood fed mosquitoes caught in the field and as well as swabbing the frogs for *Bd* at Mountain Lake Biological Station (Pembroke, VA, USA). We also conducted *Bd* contact assays, which determined whether the mosquito can acquire *Bd* from an infected surface with their legs or proboscis and transmit it to a new surface. The presence of *Bd* was confirmed using qPCR. We found *Bd* is present at MLBS, and we saw that *Cx. territans* is able to transmit *Bd* in a laboratory setting. This data brings essential insights on amphibian disease ecology and the role *Cx. territans* mosquitoes plays in vectoring pathogens and, consequently, in amphibian decline.

Investigation of the Type-B Muscarinic Acetylcholine Receptor (mAChR-B) as a Potential Insecticide Target for Control of Disease Vectors

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Effective control of disease vectors is hindered by vector populations acquiring resistance to existing insecticide modes of action. For this reason, novel modes of action are vital to sustainable vector management. Though components of the insect cholinergic system have proven to be effective targets for vector control, no commercial insecticide targets muscarinic acetylcholine receptors (mAChRs). The mAChRs are G protein-coupled receptors which function in the insect nervous system, but much remains unknown regarding their physiology and pharmacology. Three types of mAChRs are found in insects (Types-A, B, and C) with the Type-B mAChR (mAChR-B) having distinct pharmacology compared to mammalian mAChRs and the other insect mAChRs. This unique pharmacology may make the mAChR-B, which we have recently developed a selective novel pyrazole oxime against, the preferred mAChR insecticide target. While limited muscarinic molecules have been studied against the mAChR-B, we hypothesize that this subtype has unrealized potential for vector control. The physiology of the insect mAChR-B was studied using electrophysiological and *in vivo* approaches in the model organism, *Drosophila melanogaster,* which is amenable to genetic manipulation to decrease mAChR-B expression using RNA interference (RNAi). Extracellular electrophysiology indicated that pilocarpine, a non-specific agonist of mAChRs, had a biphasic affect in the *D. melanogaster* central nervous system (CNS). This biphasic affect was characterized by an increase in CNS activity at high pilocarpine concentrations and a decrease at low concentrations. Reducing expression of the mAChR-B using *in vivo* green fluorescent protein interference (iGFPi) decreased responsiveness of the CNS to pilocarpine. Additionally, toxicity of injected pilocarpine was lowered in adult *D. melanogaster* in which mAChR-B expression was targeted with iGFPi. Efforts are currently underway to express the mAChR-B in Chinese hamster ovary cells which will be used to screen established and novel small molecule libraries for chemistries acting at the mAChR-B. Collectively, these efforts have the potential to reduce human morbidity by helping develop new tools for management of disease vectors.

Evolution At Spike Position 519 In SARS-CoV-2 Facilitated Adaptation To Humans

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Three years into the COVID-19 pandemic and neither a progenitor virus to SARS-CoV-2 nor a proposed mechanism of emergence have been identified. Few residues hypothesized to be required for SARS-CoV-2 emergence have been validated experimentally and a proposed mechanism of emergence remains unknown. Identifying conserved sites which contain residues required for SARS-CoV-2 spillover and adaptation to humans would aid in the development of effective drug targets. Using this lens, we identified sites in SARS-CoV-2 which have not tolerated change during the pandemic, show signatures of strong positive selection, and have a residue that differs from all SARS-related coronaviruses infecting bat and pangolin species. We used OmegaPlus and RAiSD to detect selective sweep regions from millions of human-derived SARS-CoV-2 sequences. We identified a residue at position 519 within a sweep region in the receptor binding domain of Spike holding a static histidine in human-derived SARS-CoV-2 sequences but an asparagine in SARS-related coronaviruses identified from bats and pangolins. The Spike H519N mutant of SARS-CoV-2 displayed reduced replication in human cells, suggesting histidine at position 519 may be an important contributor towards fitness of the virus in humans. The pseudotype virus bearing 519N demonstrated significantly reduced infectivity in cells expressing the human ACE2 receptor compared to 519H. Biochemical assays indicate 519N binds significantly worse than 519H to human ACE2. Our results indicate evolution of the progenitor virus towards the histidine residue at Spike 519 was important for emergence in humans and represents a promising drug target for which small molecular inhibitors could be designed.

SPATIOTEMPORAL EMERGENCE OF LYME DISEASE IN APPALACHIA (2000-2019)

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Emerging infectious diseases are on the rise, threatening human health and straining healthcare resources. Such diseases are difficult to contain, with most characterized by rapid spread, increasing incidence, and/or increasing geographic ranges. The US Centers for Disease Control (CDC) ranks Lyme disease as the most prevalent vector-borne disease in the United States, with 30,000 reported cases and an additional estimated 400,000 unreported cases annually. At the edge of the disease's expanding range in the eastern US, there has been minimal research on the recent spread of Lyme disease in Central and Southern Appalachia, a region with quite different underlying land use and land cover features compared to the suburbs of the Northeast that are typically identified as endemic for Lyme disease. This study examines the extent of the spatiotemporal emergence of Lyme disease into and within Appalachia between 2000 and 2019 using space-time cluster analysis of the 576,406 cases reported over this period across the contiguous US, including in 423 Appalachian counties.

We collected annual county Lyme disease case data, population estimate data, and Appalachian County shape files for the years 2000-2019 from the CDC, the US Census Bureau, and the Appalachian Regional Commission databases respectively. We used SaTScan to conduct a retrospective spatial analysis of the data using Poisson scan statistic to identify both low and high Lyme disease clusters, setting the maximum cluster size at 25% of the population at risk with 999 Monte Carlo simulations. We then used ArcGIS Pro to derive choropleth maps of Lyme disease relative risk clusters. We compared the distribution of Lyme disease cases and relative risk across the contiguous US counties and within Appalachian counties and sub-regions.

We found that Lyme disease has expanded spatially over the study period. The disease has more than tripled across the contiguous US and increased more than eightfold in Appalachia, with spread southwards into and within Appalachia. These findings are important in understanding the current and future spatial range and the impacts of the continued emergence of Lyme disease in Appalachia. With this understanding, we can minimize the misdiagnosis of Lyme disease and inform public health action to reduce public vulnerability.

KEY WORDS—Lyme Disease, Vector-borne, Public Health, Spatial Analysis, Relative Risk.