



Center for  
Emerging,  
Zoonotic, and  
Arthropod-borne  
Pathogens

**OCTOBER 7, 2022**

**2022 CeZAP Infectious  
Diseases Symposium**



FRALIN LIFE SCIENCES INSTITUTE  
VIRGINIA TECH.

## 2022 CEZAP INFECTIOUS DISEASES SYMPOSIUM

7:30 – 8:30	Check In & Refreshments	Assembly Hall
<b>Morning General Session</b> <i>(Overflow seating will be available in Smithfield Room)</i>		
Session Chairs:		
XJ Meng, University Distinguished Professor, Interim Director Fralin Life Science Institute Peter Vikesland, Nick Prillaman Professor, Civil and Environmental Engineering Jonathan Auguste, Assistant Professor, Entomology		
8:30 – 8:50	<b>Welcome and Opening Remarks</b> Kylene Kehn-Hall, Director of CeZAP Dan Sui, Senior Vice President and Chief Research and Innovation Officer Aimée Surprenant, Dean of the Graduate School	Assembly Hall
8:50 – 9:00	<b>ID-IGEP Program Introduction</b> Ann Stevens, ID-IGEP Co-Director Kevin Edgar, ID-IGEP Co-Director	Assembly Hall
9:00 – 9:30	<b>Keynote</b> <i>“CDC’s Division of High Consequence Pathogens and Pathology: Recent Outbreak, Future Horizons”</i> Jennifer McQuiston Deputy Director, Division of High Consequence Pathogens and Pathology, and Manager of the 2022 CDC Multi-National Monkeypox Response Centers for Disease Control and Prevention	Assembly Hall
9:30 – 10:00	<b>Keynote</b> <i>“A Drug’s Purpose: From Erectile Dysfunction, Allergy to Hepatitis C and Covid-19”</i> Jake Liang Chief of Liver Diseases Branch, NIH Distinguished Investigator, and Member of National Academy of Medicine National Institutes of Health	Assembly Hall
10:00 – 10:30	<b>Keynote</b> <i>“Endogenous-exogenous Viral Interactions during Feline Leukemia Virus Infection”</i> Sue VandeWoude University Distinguished Professor, and Member of National Academy of Sciences Director, One Health Institute Dean of College of Veterinary Medicine and Biomedical Sciences Colorado State University	Assembly Hall
10:30 – 10:50	<b>Break</b>	
10:50 – 11:10	<b>Keynote</b> <i>“Using Language Models to Predict Zoonotic Escape in Hepatitis-E Virus”</i> T.M. Murali, Professor, Computer Sciences	Assembly Hall
11:10 – 11:30	<b>Keynote</b> <i>“Making old drugs new: Development of thermorubin and other neglected antibiotics”</i>	Assembly Hall

Andrew Lowell, Assistant Professor, Chemistry

11:30 – 11:50	<b><i>“Global Monkeypox Spread Due to Increased Air Travel”</i></b> Luis Escobar, Assistant Professor, Fish and Wildlife Conservation	Assembly Hall
11:50 – 12:10	<b><i>“Genomic diversification of <i>Listeria monocytogenes</i> isolated from natural and food-associated environments”</i></b> Jingqiu Liao, Assistant Professor, Civil Environmental Engineering	Assembly Hall
12:10 – 12:30	<b><i>“Impact of Data Structure, Availability, and Noise Distribution on Practical and Structural Identifiability of an SEIR Model”</i></b> Omar Saucedo, Assistant Professor, Mathematics	Assembly Hall
12:30- 2:30	<b>Lunch and Poster Session</b>	Latham BCDEF
12:30 – 1:30	<b>External and Internal Advisory Board Close Door Meeting</b>	Drillfield Room
2:30 – 3:30	<b>CeZAP Thematic Area Breakout Sessions</b>	

**Breakout Session #1: Antimicrobial Countermeasures (vaccine and drug)** **Assembly Hall**  
Session Chair: Zhaomin Yang, Professor, Biological Sciences

- 2:30** ***“Noble Metal Organometallic Complexes Display Antiviral Activity against SARS-CoV-2”***  
Joseph Merola, Professor, Chemistry
- 2:45** ***“Discovery of two inhibitors of the type IV pilus assembly ATPase, PilB, as antivirulence compounds”***  
Keane Dye, Postdoc
- 3:00** ***“Interactions between bacteriophage  $\chi$  and the flagellum of pathogenic *Salmonella enterica*”***  
Nathaniel Esteves, ID-IGEP
- 3:15** ***“Humoral and T-cell-mediated responses to a pre-clinical Zika vaccine candidate that utilizes a unique insect-specific flavivirus platform”***  
Danielle Porier, ID-IGEP

**Breakout Session #2: Computational Biology and Disease Modeling** **Smithfield Room**  
Session Chair: Omar Saucedo, Assistant Professor, Mathematics

- 2:30** ***“Investigating climatic influences on dengue transmission in emerging and endemic regions through mathematical modeling”***  
Michael Robert, Assistant Professor, Mathematics
- 2:45** ***“GenomeRxiv: A genome similarity-based approach for precise and accurate pathogen identification”***  
Reza Mazloom, ID-IGEP

**3:00** *“Modeling Vibrio cholera transmission risk in aquatic ecosystems using a density-based clustering algorithm”*  
Mariana Castaneda Guzman, ID-IGEP

**3:15** *“Defining “Spillover Transmission” and Future Avenues of Research”*  
Paige Van de Vuurst, ID-IGEP

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

**Duck Pond Room**

Session Chair: Cassidy Rist, Assistant Professor, Population Health Sciences

**2:30** *“Initial Findings from Two Prospective Cohort Studies in Rural Southwest Virginia: Microbiological and Chemical Contaminants in Drinking Water and Associated Health Outcomes”*

Alasdair Cohen, Assistant Professor, Population Health Sciences

**2:45** *“Genome size distributions in bacteria and archaea are strongly linked to evolutionary history at broad phylogenetic scales”*

Carolina Alejandra Martinez Gutierrez, ID-IGEP

**3:00** *“Loop-mediated isothermal amplification (LAMP) and nanopore sequencing for SARS-CoV-2 wastewater surveillance”*

Seju Kang, ID-IGEP

**3:15** *“Using metagenomics sequencing to investigate Xylella fastidiosa population and phylogeny within Virginia”*

Sahar Abdelrazek, ID-IGEP

**3:30 –3:45** **Break**

**3:45 – 4:45** **CeZAP Thematic Area Breakout Sessions**

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

**Assembly Hall**

Session Chair: Andrea Bertke, Associate Professor, Population Health Sciences

**3:45** *“Research equity in climate change and disease research”*  
Paige Van de Vuurst, ID-IGEP

**4:00** *“Histotripsy Treatment of Microbial Biofilms in Clinical Urinary Catheters”*  
Elizabeth Nowak, Faculty

**4:15** *“The Role of Sympathetic Neuronal Pathways in Regulating HSV1 and HSV2 Genital Infection”*  
Greyson Moore, ID-IGEP

**4:30** *“The Attenuation Mechanism of Interferon Gamma on Chikungunya Virus.”*  
Christina Chuong, ID-IGEP

**Breakout Session #5: Immunology and Host Pathogen Interactions**

Smithfield Room

Session Chair: Stephen Mellville, Associate Professor, Biological Sciences

- 3:45** *“Phages as commensal entities in the mammalian gut”*  
Bryan Hsu, Assistant Professor, Biological Sciences
- 4:00** *“Identification of LC3-interacting region (LIR) motifs within the NSs protein of Rift Valley fever virus as a method to discover host protein-viral protein interaction for therapeutic targeting”*  
Kaylee Petraccione, ID-IGEP
- 4:15** *“Host interactors of the E1 glycoprotein of Venezuelan equine encephalitis virus as targets for antiviral Drugs”*  
Lauren Panny, ID-IGEP
- 4:30** *“Development of animal models for the study of Cache Valley Virus”*  
Krisangel López, ID-IGEP

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

Duck Pond Room

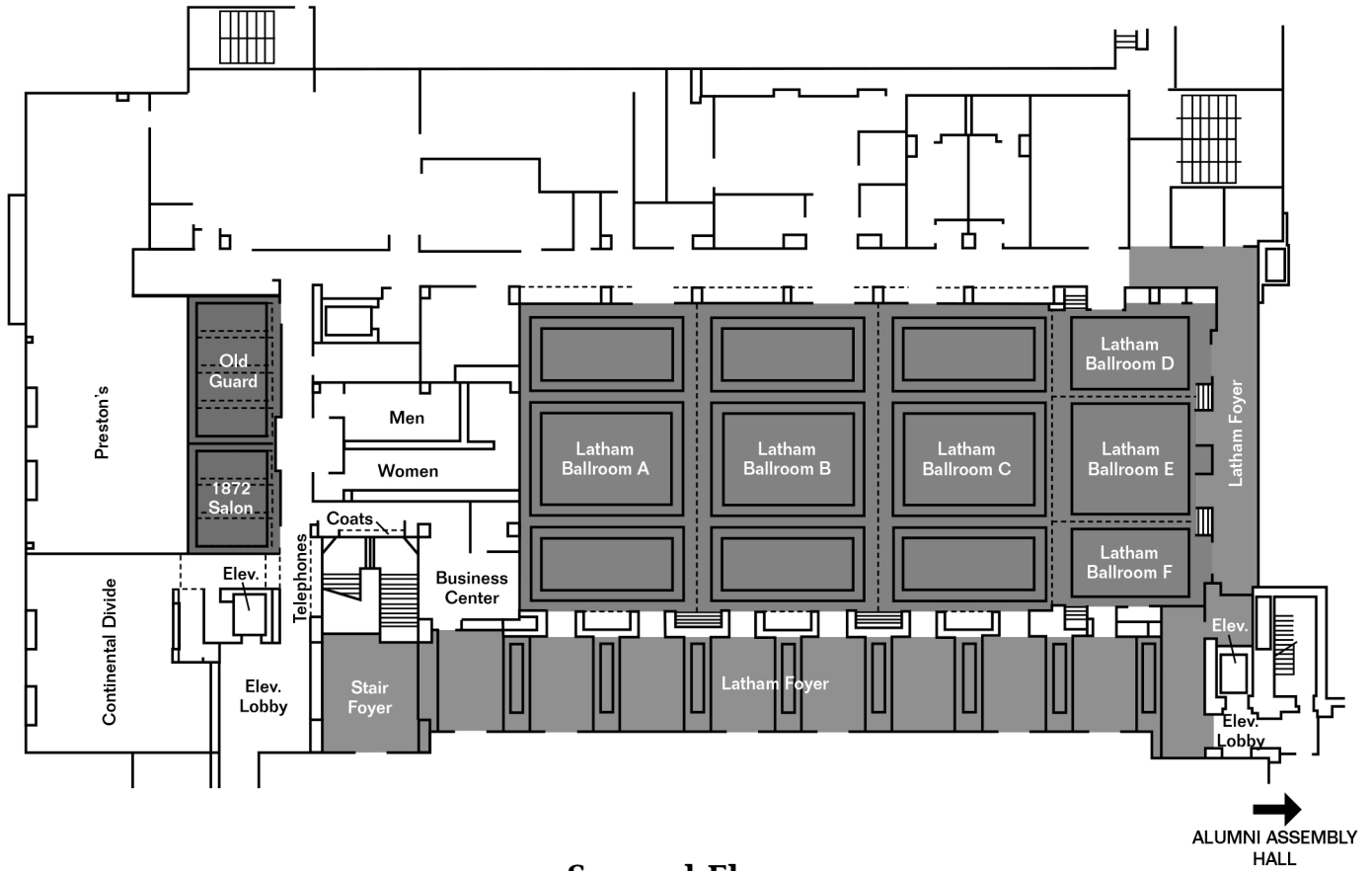
Session Chair: John Maurer, Professor, Animal and Poultry Sciences

- 3:45** *“Sex and habitat drive hantavirus prevalence in marsh rice rat populations impacted by the Deepwater Horizon oil spill”*  
Anna Perez-Umphrey, ID-IGEP
- 3:57** *“Enzymes responsible for the hydrolysis of fatty acids from exogenous lysophosphatidylcholine by asexual Plasmodium falciparum”*  
Jiapeng Liu, ID-IGEP
- 4:09** *“Multi-threaded approach to understanding links between mosquito ecology and hostseeking Behavior”*  
Karthikeyan Chandrasegaran, Postdoc
- 4:21** *“Seasonality of three human-biting ixodid ticks in western Virginia”*  
Alexandra Cumbie, Postdoc
- 4:33** *“Modeling the effects of environmental temperature on vectors of African trypanosomiasis”*  
Paul Huxley, Postdoc

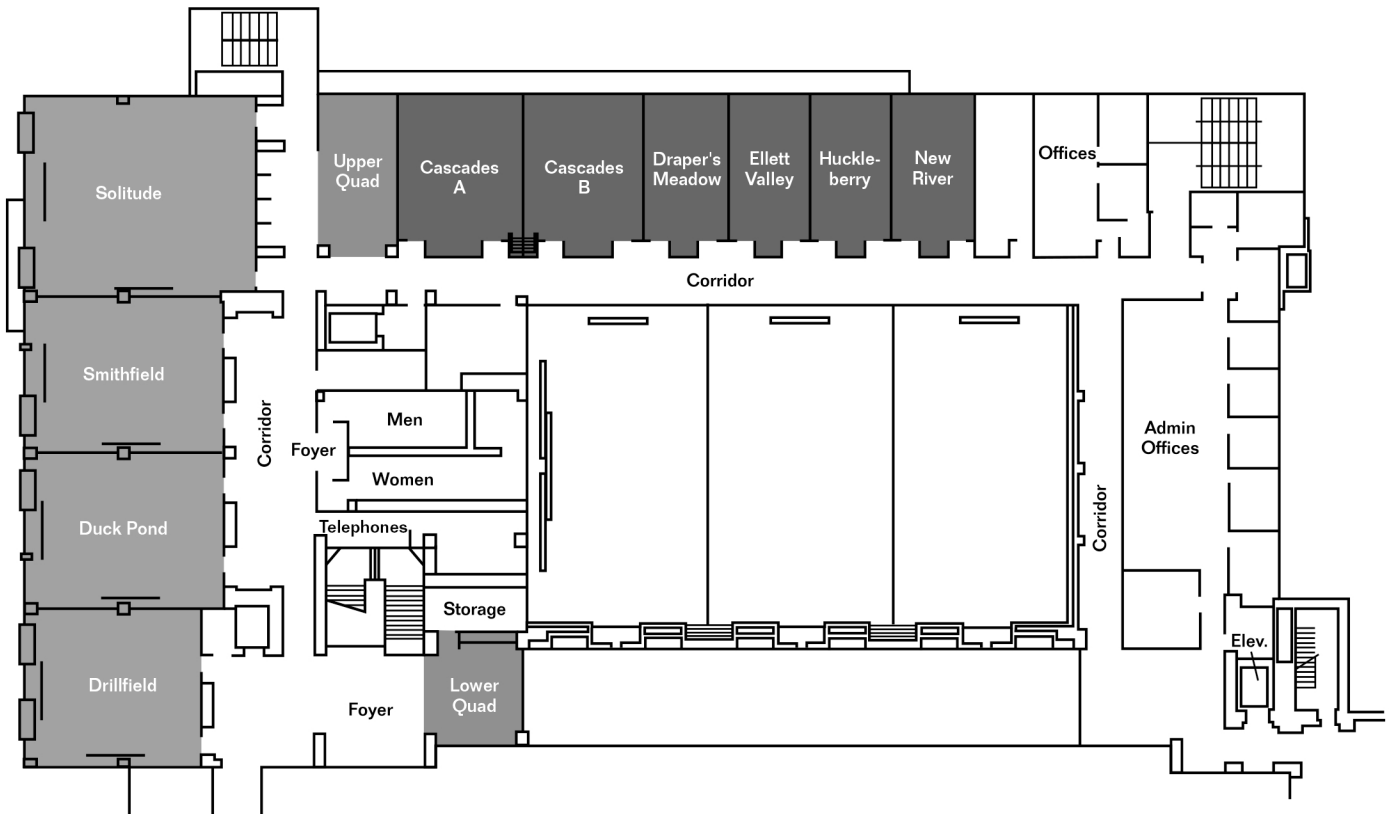
**4:45 – 5:00** Break**5:00 – 5:30** Award Reception and Closing Remarks

Assembly Hall

# First Floor



# Second Floor





Center for Emerging, Zoonotic,  
and Arthropod-borne Pathogens

# Abstracts

Oral Presentations



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## Morning Session

### TM Murali

Professor, Department of Computer Science  
College of Engineering

### **"Using Language Models to Predict Zoonotic Escape in Hepatitis-E Virus "**

Andrew Chan Department of Computer Science, Virginia Tech, [andrewclchan211@vt.edu](mailto:andrewclchan211@vt.edu)

T. M. Murali Department of Computer Science, Virginia Tech, [murali@cs.vt.edu](mailto:murali@cs.vt.edu)

Hepatitis-E virus (HEV) causes more than 20 million human infections with 40,000 deaths annually. It is known to infect more than a dozen animal hosts. It is a zoonotic pathogen with more than a dozen animal hosts. However, the viral genetic element(s) that are responsible for allowing the virus to jump from animal to human hosts and to adapt to humans remain unknown. Obtaining a clear understanding of how zoonotic escape occurs could aid in therapeutic development and mitigation of future outbreaks.

Given a collection of full-length HEV genomes and their genotypes and hosts, we sought to build a machine learning model that could predict the host and genotype for a previously-unseen sequence. Our eventual goal is to use this model to determine which sequences in the virus may be responsible for human infection.

We took advantage of recent, powerful developments in natural language processing (NLP) models. In particular, a recent paper used a bidirectional long short-term memory (BiLSTM) model to learn the language of viral sequence evolution and escape [1]. Their intuition was that a "grammatically-correct" sequence would be recognized by the immune system where an ungrammatical sequence was capable of immune escape.

We adapted this methodology to our problem. We used a BiLSTM-based model on HEV protein sequences to learn an embedding of each sequence into a multi-dimensional vector space. We optimized the parameters of the model by training it by removing each character in a sequence and trying to predict it as accurately as possible. We further trained the model using a multi-layer perceptron to predict a sequence's host or genotype. We compared this strategy to another with only the second level of training.

We downloaded 905 complete genomes for *Orthohepevirus A (Paslahepevirus balayani)*. Over 29.8% of the genomes had "Unknown" hosts. Therefore, we directly queried GenBank for HEV sequences, parsed the entries, and supplemented these efforts with examination of the corresponding papers. These steps enabled us to identify the hosts for as many as 92% of the genomes. As many as 80% of the sequences in our data were from human hosts. The second-most common host was swine at 13%. Next, due to nonstandard genotype submissions in GenBank, we used the "Hepatitis E Virus Automated Genotyping Tool" to identify the genotype of each sequence with well established reference sequences [2]. From each the 905 complete genomes, we used the amino acid sequences of the relevant open reading frames (ORF1, ORF2, ORF3, ORF4) as input to our model.

We split these data in a 1:1 ratio between training and testing sets. We achieved an accuracy of 84.83% and an area under the receiver-operator characteristic curve (AUROC) of nearly 100% on the test data with the two-stage method but only 81% and 50% respectively with the one stage method. Note that a random predictor will obtain an accuracy of 80% and an AUROC of 50%.

In ongoing research, we are applying the classifier to predict the genotype and to improve the accuracy of the classifier. We are also seeking to make the classifier interpretable so that we can identify HEV genetic elements that may be associated with zoonosis.

**Acknowledgments.** We thank Dr. X. J. Meng and Dr. Bo Wang for many valuable discussions.

#### **References**

[1] B. Hie, E. D. Zhong, B. Berger, and B. Bryson. Learning the language of viral evolution and escape. *Science*, 371(6526):284–288, 01 2021.

[2] D. B. Smith, P. Simmonds, J. Izopet, E. F. Oliveira-Filho, R. G. Ulrich, R. John, M. Koenig, S. Jameel, T. J. Harrison, X. J. Meng, H. Okamoto, W. H. M. Van der Poel, and M. A. Purdy. Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol*, 97(3):537–542, Mar 2016.



## Morning Session

### Andrew Lowell

Assistant Professor, Department of Chemistry  
College of Science

### "Making Old drugs new: Development of thermorubin and other neglected antibiotics"

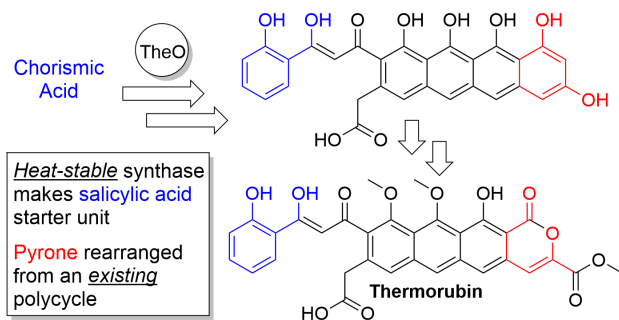
Andrew N. Lowell, Jennifer P. McCord, Zachary A. Kohanov, Cole Gannett

Antimicrobial resistance (AMR) poses a major threat to human health, a problem accentuated by the lack of new antibiotic discovery over the past several decades. One rich source of potential antibiotics is the many antimicrobial natural products that were previously identified but remain undeveloped. This category of neglected antibiotics is surprisingly large with follow-up ranging from structurally unknown isolates to well-characterized molecules with high activity and new methods of inhibition.

One such antibiotic is thermorubin, a tetracyclic naphthoisocoumarin natural product that demands investigation due to its novel mechanism of protein synthesis inhibition and its unusual structural features. It suppresses an established target, the bacterial ribosome, but does so in an unusual manner: by binding tightly to the assembled ribosome complex rather than either subunit individually. This mode of action is hypothesized to prevent dissociation of the ribosomal subunits, interfering with initiation of protein synthesis and ribosome recycling. Development and optimization of thermorubin would add a valuable new antibiotic to our existing arsenal, one that is not inhibited by current resistance mechanisms.

In this talk, we describe the identification of the biosynthetic cluster responsible for thermorubin from the sequenced *Laceyella sacchari* producer species and its confirmation via heterologous expression in *E. coli*. Based on an in-depth annotation of the cluster, we propose a biosynthetic pathway that accounts for the formation of the unique, non-terminal pyrone. Additionally, expression and use of salicylate synthase TheO enabled testing of the stability properties of this extremophile-derived enzyme. TheO displayed rapid kinetics and a remarkably robust secondary structure, converting chorismate to salicylate with a  $K_M$  of  $109 \pm 12 \mu\text{M}$ ,  $k_{\text{cat}}$  of  $9.17 \pm 0.36 \text{ min}^{-1}$ , and catalytic efficiency ( $k_{\text{cat}}/K_M$ ) of  $84 \pm 9 \text{ nM}^{-1} \text{ min}^{-1}$  and retained significant activity up to  $50^\circ\text{C}$ .

Beyond thermorubin, many other antimicrobials with broad-spectrum activity are known but were never developed for clinical use. These may prove a rich potential source of new antibiotics if they can be semi-synthetically derivatized to narrow their activity. Toward developing thermorubin and other neglected antibiotics, we are working collaboratively with the Brown group to computationally learn about how antibiotics and broad-spectrum inhibitors bind to the ribosome as well as synthesizing and testing antibiotic derivatives in our lab using medicinal chemistry and antimicrobial assays.



## Morning Session

### Luis Escobar

Assistant Professor, Department of Fish and Wildlife Conservation  
College of Natural Resources and Environment

### ***"Global Monkeypox Spread Due to Increased Air Travel"***

Luis E. Escobar, DVM, PhD.

Department of Fish and Wildlife Conservation and Center for Emerging Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA, United States

Huijie Qiao,

PhD Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Monkeypox outbreaks have been reported in 91 countries (May-August, 2022), encompassing all continents except Antarctica. There have been a total of 41,304 confirmed monkeypox cases reported, and 89% of affected countries without historically reported monkeypox outbreaks. International air travel in the summer of 2022, after the reduction of COVID-19 mobility restrictions, surpassed pre-pandemic levels of air traffic. Air travel was recovered for most countries (~79%) and exceeded by some countries. Surpassed air traffic is a strong predictor of monkeypox cases in non-endemic countries. The current monkeypox epidemic has clear geographical patterns, which can help inform its prevention, control, and epidemiological surveillance. Air passenger flow is a predictor of hotspots areas of monkeypox spread, which suggests a potential role of international airports as first front to monitor risk of disease emergence.

## Morning Session

### Jingqiu Liao

Assistant Professor, Department of Civil and Environmental Engineering  
College of Engineering

#### **"Genomic diversification of *Listeria monocytogenes* isolated from natural and food-associated environments"**

Jingqiu Liao<sup>1, 2#</sup>, Xiaodong Guo<sup>2</sup>, Daniel Weller<sup>2</sup>, Genevieve Sullivan<sup>2</sup>, Martin Wiedmann<sup>2</sup>

<sup>1</sup>Department of Civil and Environmental Engineering, Virginia Tech, VA, USA. <sup>2</sup>Department of food science, Cornell University, Ithaca, NY, USA.

#Address correspondence to Jingqiu Liao, [liaoj@vt.edu](mailto:liaoj@vt.edu)

*Listeria monocytogenes*, a leading cause of mortality and morbidity due to foodborne illnesses, is ubiquitously prevalent in natural environments, yet little is known how likely it transmits from natural environments to humans via the food supply chain. Answering this question requires a better understanding of the ecological mechanisms underlying the genetic variability of *L. monocytogenes*. To this end, we compared whole genome sequence data for 177 *L. monocytogenes* isolates from the soil in the natural environment with 176 isolates from produce processing facilities. We found that the phylogenetic and genomic profiles of *L. monocytogenes* isolates strongly differ by the environment, as supported by environment-associated subclades as well as by the differential presence of plasmids, stress islands, and orthologous genes encoding functions enriched for cell envelope biogenesis. We further identified potential selective pressures driving the genome-environment association in soil-dwelling *L. monocytogenes* isolates. These pressures include soil property, climate and bacterial species chiefly representing *Actinobacteria* and *Proteobacteria*. Collectively, our data suggest that the genomic variability in *L. monocytogenes* is associated with local adaptation to different environments, which may limit their transmission from natural to food-associated environments.

## Morning Session

### Omar Saucedo

Assistant Professor, Department of Mathematics  
College of Science

### ***"Impact of Data Structure, Availability, and Noise Distribution on Practical and Structural Identifiability of an SEIR Model"***

Lale Asik, Amanda Laubmeier, Benjamin Levy, Omar Saucedo, Tim Pollington, Olivia Prosper, Tingting Tang

With the increasing practice of using biological data to assess and parameterize theoretical models, it is important to understand the conditions under which we can reliably recover model parameters from available data. This is particularly important in the context of epidemiological data, which may gradually become available alongside an emerging infection or may only be reported over large time intervals. In this work, we consider an SEIR infection model, with three unknown parameters. Our goal is to assess how different methods and resolutions of data collection determine parameter identifiability. We consider the impact of the frequency and duration of observations, for different types of observed data (infections, incidence, and cumulative incidence). We utilize both Monte Carlo simulations and correlation matrices to assess parameter identifiability under these conditions.

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

## Joseph Merola

Professor, Department of Chemistry  
College of Science

### "Noble Metal Organometallic Complexes Display Antiviral Activity against SARS-CoV-2"

Christina Chuong<sup>1</sup>, Christine M. DuChane<sup>2</sup>, Emily M. Webb<sup>3</sup>, Pallavi Rai<sup>1</sup>, Jeffrey M. Marano<sup>4</sup>, Chad M. Bernier<sup>2</sup>, Joseph S. Merola<sup>2,\*</sup> and James Weger-Lucarelli<sup>1,\*</sup>

<sup>1</sup> Department of Biomedical Sciences and Pathobiology, Virginia Tech, VA-MD Regional College of Veterinary Medicine, Blacksburg, VA 24061, USA; cc6re@vt.edu (C.C.); pallavirai@vt.edu (P.R.)

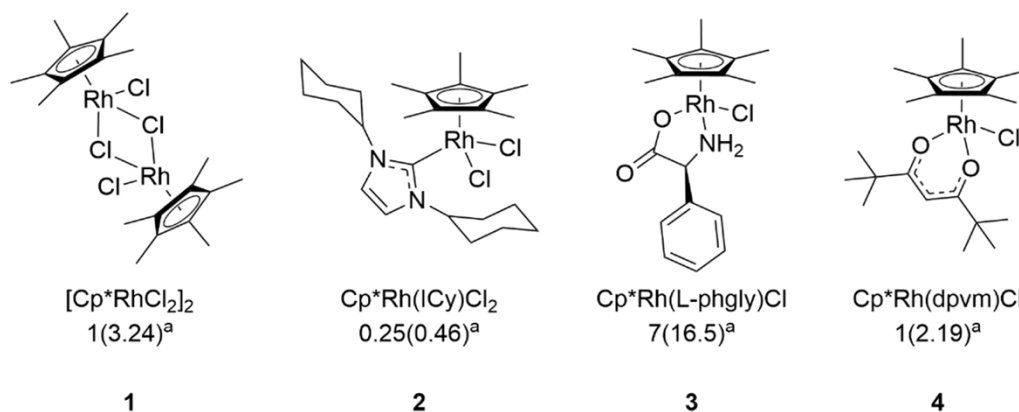
<sup>2</sup> Department of Chemistry, Virginia Tech, Blacksburg, VA 24061, USA; cduchane@vt.edu (C.M.D.); chadb@vt.edu (C.M.B.)

<sup>3</sup> Department of Entomology, Virginia Tech, Blacksburg, VA 24061, USA; wemily2@vt.edu

<sup>4</sup> Department of Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA 24061, USA; jmarano@vt.edu

\* Correspondence: jmerola@vt.edu (J.S.M.); weger@vt.edu (J.W.-L.)

**Abstract:** SARS-CoV-2 emerged in 2019 as a devastating viral pathogen with no available preventative or treatment to control what led to the current global pandemic. The continued spread of the virus and increasing death toll necessitate the development of effective antiviral treatments to combat this virus. To this end, we evaluated a new class of organometallic complexes as potential antivirals. Our findings demonstrate that two pentamethylcyclopentadienyl (Cp\*) rhodium piano stool complexes, Cp\*Rh(1,3-dicyclohexylimidazol-2-ylidene)Cl<sub>2</sub> (complex **2**) and Cp\*Rh(dipivaloylmethanato)Cl (complex **4**), have direct virucidal activity against SARS-CoV-2. Subsequent in vitro testing suggests that complex **4** is the more stable and effective complex and demonstrates that both **2** and **4** have low toxicity in Vero E6 and Calu-3 cells. The results presented here highlight the potential application of organometallic complexes as antivirals and support further investigation into their activity.



Organometallic complexes investigated for virucidal activity against SARS-CoV-2. Minimum inhibitory concentration given below each compound in mg/mL (mM)

**Keywords:** SARS-CoV-2; COVID-19; organometallics; metallodrugs; antiviral; virucidal

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

### Keane Dye

Postdoc, Department of Biological Sciences

Faculty Mentor: Zhaomin Yang

### ***“Discovery of two inhibitors of the type IV pilus assembly ATPase, PilB, as antivirulence compounds”***

With the pressing antibiotic resistance pandemic, antivirulence has been increasingly explored as an alternative strategy against bacterial infections. The bacterial type IV pilus (T4P) is a well-documented virulence factor and attractive target for small molecules for antivirulence purposes. The PilB ATPase is essential for T4P biogenesis because it catalyzes the assembly of monomeric pilins into the polymeric pilus filament. In our work we describe the identification of two PilB inhibitors by a high throughput screen (HTS) *in vitro* and their validation as effective inhibitors of T4P assembly *in vivo*. We used *Chloracidobacterium thermophilum* PilB as a model enzyme to optimize an ATPase assay for the HTS. From a library of 2,320 compounds, benserazide and levodopa, two FDA-approved drugs for Parkinson’s disease, were identified and confirmed to be PilB inhibitors biochemically. We demonstrate that both compounds inhibited the T4P-dependent motility of the bacteria *Myxococcus xanthus* and *Acinetobacter nosocomialis*. Benserazide and levodopa were shown additionally to inhibit *A. nosocomialis* biofilm formation, a T4P-dependent process in this bacterium. Using *M. xanthus* as a model, both compounds were shown to inhibit T4P assembly in a dose-dependent manner. These results suggest that these two compounds from this screen are effective against the PilB protein *in vivo*. The potency of benserazide and levodopa as PilB inhibitors both *in vitro* and *in vivo* demonstrate potentials of the HTS and its two hits here for the development of anti-T4P chemotherapeutics.

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

### Nathaniel Esteves

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Birgit Scharf

### ***"Interactions between bacteriophage X and the flagellum of pathogenic Salmonella enterica"***

Nathaniel C. Esteves and Birgit E. Scharf

Flagellotropic (flagellum-dependent) phages are viruses which begin their infection process by attaching to the flagellar filament of their respective host cells, using the rotation to reach the cell surface. This results in the unique requirement of motility for phage infection. Due to the fact that motility is a significant virulence factor for many species of pathogenic bacteria, including *Salmonella enterica*, flagellotropic phages may be particularly effective as antimicrobial agents. Flagellotropic phages force an exploitable evolutionary tradeoff: a bacterial cell which represses motility to avoid infection by a flagellotropic phage would likely also attenuate its own virulence. Bacteriophage  $\chi$  is a flagellotropic phage which infects three species of potentially pathogenic organisms: *Salmonella enterica*, *Escherichia coli*, and *Serratia marcescens*. The interactions between phage and flagellum are complex and poorly understood. It is well known that  $\chi$  requires a motile host, and that even intact paralyzed flagella do not allow any infection. The long tail fiber of  $\chi$  phage is thought to wrap itself into the grooves of the flagellar filament formed by the monomers of flagellin. At this point, the rotation of the filament pulls the phage to the cell surface like a nut moving down a bolt. However, the nuances of this interaction are understudied, as are the reasons why the flagella of some serovars of *Salmonella* allow  $\chi$  phage adsorption while others are entirely resistant to phage binding. Using targeted and random mutagenesis, we have determined specific domains within flagellin that are directly involved in  $\chi$  phage attachment, beginning to elucidate the factors determining the host range of  $\chi$ . With further knowledge about interactions between flagella and the phage tail fiber, coupled with  $\chi$  phage's naturally broad host range, it may be possible in the future to genetically engineer host range mutants which could be tailored to only infect certain strains of motile pathogens.

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

### Danielle Porier

ID IGEP affiliated student, Entomology

Faculty Mentor: Jonathan Auguste

### ***"Humoral and T-cell-mediated responses to a pre-clinical Zika vaccine candidate that utilizes a unique insect-specific flavivirus platform"***

Danielle L. Porier<sup>1</sup>, Manette Tanelus<sup>1</sup>, William B. Stone<sup>1</sup>, Krisangel Lopez<sup>1</sup>, Dawn I. Auguste<sup>1</sup>, Albert J. Auguste<sup>1,2</sup>

<sup>1</sup>Department of Entomology, College of Agriculture and Life Sciences, Virginia Tech, Blacksburg, VA 24061, USA; <sup>2</sup>Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA 24061, USA.

Vaccination is critical for the control and prevention of viral outbreaks, yet conventional vaccine platforms may involve trade-offs between vaccine immunogenicity and safety. Insect-specific flaviviruses (ISFVs) are emerging as a novel method to overcome this challenge. ISFVs are safe; they neither replicate nor cause disease in vertebrates, and hence also don't require traditional inactivation methods that can result in antigenic degradation. Previously, we used a novel ISFV called Aripo virus (ARPV) to create a promising recombinant Zika virus (ZIKV) vaccine candidate (called ARPV/ZIKV) that consists of ZIKV precursor membrane and envelope (prM-E) genes expressed on an ARPV backbone. Previously, ARPV/ZIKV showed no pathogenicity, including in suckling mice injected intracranially. Immunocompetent and immunocompromised mice immunized with a single unadjuvanted dose were also completely protected from lethal ZIKV challenge. Given ZIKV's propensity to evade key innate antiviral responses in humans, and in order to assess correlates of protection and adjuvant selection in the future, a better understanding of the balance between vaccine-induced humoral and T-cell responses is required. Here, we explore these responses for both developing immunity post-immunization and for providing protection post-challenge. Passive transfer of antibodies from ARPV/ZIKV-immunized mice to naïve IFNAR<sup>-/-</sup> mice prior to challenge emphasized neutralizing antibodies as an important correlate of protection. However, circulating antibodies post-transfer were not sufficient for full protection. Follow-up *in vivo* T-cell depletion studies of CD8<sup>+</sup> or CD4<sup>+</sup> T-cells in IFNAR<sup>-/-</sup> mice at the time of challenge indicated the potential importance of vaccine-induced T-cell responses for protection. Vaccine efficacy studies in Rag1 KO, Tcra KO, and muMt<sup>-/-</sup> mice also demonstrated the importance of T-cell responses for developing immunity after ARPV/ZIKV immunization. Overall, ARPV/ZIKV induces robust responses in both branches of the adaptive immune system, meaning that ISFV platforms continue to be a promising method for vaccine development.



## **Breakout Session #2: Computational Biology and Disease Modeling**

### **Michael Robert**

Assistant Professor, Department of Mathematics

#### ***" Investigating climatic influences on dengue transmission in emerging and endemic regions through mathematical modeling"***

Dengue, a virus transmitted by the mosquito species *Aedes aegypti*, has been spreading in temperate regions across the globe, and outbreaks in more tropical endemic regions have increased in severity in recent years. This spread and increase in transmission has been driven by numerous factors including higher temperatures and more erratic precipitation patterns caused in part by global climate change. Temperature and precipitation impact various parts of the dengue transmission cycle, including mosquito development and survival and the incubation period of the virus in the mosquito. With the continuing threat of climate change, it is critical that we develop a better understanding of meteorological influences on the spread of dengue. Here, we employ a mathematical model to explore the effects of seasonal temperature variation on the potential for dengue transmission in regions where dengue is emerging as well as in endemic regions. As case studies, we explore recent dengue emergence in temperate areas of Argentina and recent increases in dengue transmission in the Dominican Republic. Temperate areas of Central Argentina experienced their first dengue outbreaks in 2009 and have since experienced yearly transmission and four large outbreaks. The Dominican Republic, where dengue has long been endemic, has reported significantly larger outbreaks in the last five years. With our model, we show how seasonal and diurnal temperature patterns influence risk of dengue transmission, and how warming temperatures may exacerbate this risk. We highlight the importance of our findings for endemic and emerging populations, and discuss the potential implications of our results for mosquito control and dengue mitigation strategies.

## Breakout Session #2: Computational Biology and Disease Modeling

### Reza Mazloom

ID IGEP affiliate student

### ***"GenomeRxiv: A genome similarity-based approach for precise and accurate pathogen identification"***

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Fast, precise, and accurate pathogen identification is key to successful control of disease outbreaks and prevention of epidemics and pandemics. While it is possible to use pathogen-specific assays, such as PCR, to detect known pathogens, there are no assays available to identify yet unknown, newly emerging pathogens. Therefore, sequencing all microbes associated with a symptomatic organism, i.e., metagenomic sequencing, is a promising approach. To do this, a large sequence database of all known microbes and fast algorithms to compare query sequences with the database sequences are required. We previously developed the Life Identification Number (LIN) system for genome-based classification and identification. The LIN system provides a framework to organize all microbial genome sequences by their similarity. While most taxonomic genome databases of prokaryotes only reach the resolution of species, the LIN system approaches strain-level resolution by extending the use of average nucleotide identity (ANI) from the established 95% species threshold to 99.975%. We implemented LINs as part of the LINbase web server for genome-based bacterial pathogen identification, which we are currently upgrading to a type of genome preprint service, called genomeRxiv. Both services use a computationally efficient k-mer-based approach for faster identification while increasing the number of genomes in the database. We are also expanding the LIN approach to fungal pathogen identification and combining the popular Kraken2 tool, used for taxonomic characterization of metagenomes, with the LIN approach to facilitate identification with strain-level resolution. We will show examples of how the described approaches and tools can contribute to faster curate identification of emerging plant, animal, and human pathogens.

## Breakout Session #2: Computational Biology and Disease Modeling

### Mariana Castaneda Guzman

ID IGEP affiliated student, Fish and Wildlife Conservation

Faculty Mentor: Luis Escobar

### ***"Modeling Vibrio cholera transmission risk in aquatic ecosystems using a density-based clustering algorithm"***

Most infectious diseases in animals do not occur randomly. Instead, diseases in livestock and wildlife are predictable in terms of the geography, time and species affected. This, in turn, allows to quantify and trace specific environmental conditions associated with disease occurrence. Machine learning modeling is an analytical tool recently employed in spatial epidemiology to map vector-borne disease transmission, accounting for multivariate environmental conditions. Nevertheless, understanding the effects of biotic and abiotic environmental conditions on the distribution and abundance of environmentally transmitted diseases (i.e., non-vector-borne) remains in its infancy. Inadequate availability of analytical methods that analyze biotic and abiotic variables limits capacities to anticipate where directly transmitted diseases can emerge. To solve this problem, I propose an adaptation of the density-based spatial clustering algorithm based on ecological theory to model relationships between biotic and abiotic environmental conditions as predictors of *Vibrio cholerae*, a water-borne/food-borne pathogen. This study focused on using classical ecological niche theory to implement a density-based clustering algorithm, i.e., density-based spatial clustering of applications with noise (DBSCAN), to predict the spatial distribution of *V. cholerae* in seawaters globally. This method may provide opportunities to use biotic and abiotic data to understand how global changes affect water-borne infectious diseases globally without the need for a priori assumptions of the shape of the response of organisms to biotic factors. More specifically, I propose a revised version of the *Marble* algorithm, an adaptation of DBSCAN for realized ecological niche estimates. The assessment demonstrates that *Marble* can accurately reconstruct and predict the global distribution of *V. cholerae*. As such, *Marble* can be used to estimate the distribution of emerging infectious diseases in aquatic ecosystems using presence-only data, accounting for abiotic and biotic variables, and without the need for delimitation of study areas.

## Breakout Session #2: Computational Biology and Disease Modeling

### Paige Van de Vuurst

ID IGEP affiliated student, Fish and Wildlife Conservation

Faculty Mentor: Luis Escobar

### ***"Defining "Spillover Transmission" and Future Avenues of Research"***

**Authors:** Luis E Escobar<sup>1,2,3,4,5\*</sup>, Andres Velasco-Villa<sup>6</sup>, Panayampalli S. Satheshkumar<sup>6</sup>, Yoshinori Nakazawa<sup>6</sup>, Paige Van de Vuurst<sup>1,2</sup> \*

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\*Speaker

The term 'spillover' is often used to refer to the transmission of a parasitic agent or pathogen from its primary reservoir host to a new, naïve yet susceptible and permissive host species. Pathogens usually do not cause intense negative impacts in their primary reservoir hosts due to a long shared evolutionary history, where pathogens generally undergo low-high virulence trade-offs within their hosts until they reach maximum fitness at a usually low virulence equilibrium. Nevertheless, spillover transmission of these pathogens to other animal species can result in the onset of disease or death to the new host. Spillover transmission can result in unsuccessful transmission among co-specifics (i.e., pathogen die off before propagation) or successful establishment of the pathogen in the host species with consequent secondary transmission among co-specifics and other sympatric susceptible species. As such, spillover transmission is a common yet critical event in nature where a primary host and a range of susceptible species coincide spatially and temporally. These conditions can result in disease emergence. Uncertainties surrounding spillover chances and the impact of different ecological drivers across scales have, however, limited the current understanding of this phenomenon and precluded accurate predictions of disease emergence. Rabies virus (RABV) is a well-documented pathogen which still inflicts heavy impact over humans, companion animals, wildlife, and livestock throughout Latin America due to substantial spatial temporal and ecological—natural and expansional—overlap with several virus reservoir hosts. The common vampire bat has become the most common reservoir host for RABV in the tropical and subtropical Americas, thus functioning as a major spillover agent transmitting RABV to a great number of sympatric susceptible species. Thereby, this disease system represents a robust avenue through which the uncertainties surrounding spillover transmission may be elucidated. The continued study of spillover transmission necessitates more research on the cross-scale impacts of ecological forces linked to the propensity of spillover success. Identifying the quantitative and qualitative factors that facilitate spillover would allow better disease forecasting and would prevent public health impacts on those most at risk populations across the globe.

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

### Alasdair Cohen

Assistant Professor, Department of Population Health Sciences,

### ***"Initial Findings from Two Prospective Cohort Studies in Rural Southwest Virginia: Microbiological and Chemical Contaminants in Drinking Water and Associated Health Outcomes"***

Alasdair Cohen, Md Rasheduzzaman, Leigh-Anne Krometis, Marc Edwards, Amanda Darling, Elizabeth Rogawski-McQuade, Suporn Pholwat, Teresa Brown, Beth O'Connell, Mami Taniuchi

**BACKGROUND:** Consumption of unsafe drinking water is associated with a substantial burden of disease globally. In the US, ~1.8 million people in rural areas lack reliable access to safe drinking water, a problem that is particularly acute in many rural areas of Appalachia. However, our understanding of which regions and populations have higher risks of exposure to contaminated water is hampered by a lack of data, and even less is known about water-associated health impacts.

**OBJECTIVES:** Our objective was to better understand potential exposures to microbiological and chemical contaminants in drinking water by assessing the use and quality of primary household-level drinking water sources as well as associated health outcomes, poverty-related indicators, and sociocultural factors in lower-income households without utility-supplied water in rural southwest Virginia.

**METHODS:** In 2021, with support from the Wise County public water and sewer utility in southwest Virginia, we initiated a prospective cohort study open to all households in a small, lower-income, rural community the utility was considering for a water supply extension project. In 2022, in collaboration with the non-profit Appalachia Service Project (ASP), we initiated a prospective cohort study in Lee and Wise Counties open to households ASP had recently served or was planning to offer services to in the summer of 2022. We collected survey data (face-to-face interviews) and multiple sets of water samples (tap, source, and bottled water) from consenting households. Field measurements were taken for pH, temperature, conductivity, and dissolved oxygen. Water samples were tested for *E. coli*, total coliforms, nitrate, sulfate, heavy metals such as arsenic, cadmium, and lead, and for 30+ specific enteric pathogens. For the ASP study, water samples were also tested for free chlorine and disinfection byproducts, and saliva swabs were collected from consenting household members. Our studies were approved by Virginia Tech's IRB (#21-763 and #22-293).

**(INITIAL) RESULTS:** Among the 69% (n=9) eligible and non-vacant homes that agreed to participate at baseline for the 2021 study, all had piped well water, though 67% (n=6) used bottled water as their primary source of drinking water. The majority of household (67%, n=6) reported incomes of <\$43,000/year. Total coliforms were detected in samples from 44.4% (n=4) of households, *E. coli* was detected in one home, and enteric pathogens (*Aeromonas*, *Campylobacter*, *Enterobacter*) were detected in 33% (n=3) of homes. Tap water samples from 44% (n=4) of homes exceeded ½ the US EPA MCL for nitrate, and 56% (n=5) exceeded ½ the US EPA SMCL for iron. Sodium concentrations in source water samples ranged from 3.3 – 110.0 mg/L (mean=66.0, SD=36.0, median=76.4, n=9). Reported diarrhea was 25% more likely in homes with measured *E. coli* and/or specific pathogens (Risk Ratio=1.25, cluster-robust standard error=1.64, p=0.865). For the 2022 ASP study, at this writing 33 eligible households participated in baseline data collection, conducted in July 2022, and data analyses are underway.

**BROADER SIGNIFICANCE:** Our initial findings indicate that microbiological and/or chemical contamination in drinking water is not uncommon for lower-income residents without utility-supplied water in rural areas of southwest Virginia, and that many, if not most, such rural households rely on bottled water as their primary source of drinking water. Our detection of specific enteric pathogens and relatively high concentrations of contaminants such as nitrate and iron in water samples indicates that additional research and surveillance data are needed to better understand which regions, communities, and populations in Central Appalachia may be exposed to contaminated water sources, the nature and extent of associated adverse health outcomes, and what interventions might be implemented to expand safe water access.

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

### **Carolina Alejandra Martinez Gutierrez**

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Frank Aylward

### ***“Genome size distributions in bacteria and archaea are strongly linked to evolutionary history at broad phylogenetic scales”***

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The evolutionary forces that determine genome size in bacteria and archaea have been the subject of intense debate over the last few decades. Although the preferential loss of genes observed in prokaryotes is explained through the deletional bias, factors promoting and preventing the fixation of such gene losses often remain unclear. Importantly, statistical analyses on this topic typically do not consider the potential bias introduced by the shared ancestry of many lineages, which is critical when using species as data points because of the potential dependence on residuals. In this study, we investigated the genome size distributions across a broad diversity of bacteria and archaea to evaluate if this trait is phylogenetically conserved at broad phylogenetic scales. After model fit, Pagel’s lambda indicated a strong phylogenetic signal in genome size data, suggesting that the diversification of this trait is influenced by shared evolutionary histories. We used a phylogenetic generalized least-squares (PGLS) analysis to test whether phylogeny influences the predictability of genome size from dN/dS ratios and 16S copy number, two variables postulated to play a role in shaping genome size. These results confirm that failure to account for evolutionary history can lead to biased interpretations of genome size predictors. Overall, our results indicate that although bacteria and archaea can rapidly gain and lose genetic material through gene transfer and deletions, respectively, phylogenetic signal for genome size distributions can still be recovered at broad phylogenetic scales that should be taken into account when inferring the drivers of genome size evolution.

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

### **Seju Kang**

ID IGEP affiliated student, Civil and Environmental Engineering

Faculty Mentor: Peter Vikesland

### ***"Aloop-mediated isothermal amplification (LAMP) and nanopore sequencing for SARS-CoV-2"***

Loop-mediated isothermal amplification (LAMP) and nanopore sequencing for SARS-CoV-2 wastewater surveillance

Wastewater-based surveillance (WBS) has gained attention as a strategy to provide early warning of disease outbreaks. WBS relates viral loads measured in wastewater to fecal shedding of SARS-CoV-2 by infected members of the population served by a sewershed. While PCR-based techniques are the most reliable platforms for detection of SARS-CoV-2, they require centralized facilities and professional personnel, thus limiting their capacity. We evaluated the potential for reverse-transcription loop-mediated isothermal amplification and nanopore sequencing (LAMPore) as a feasible assay for COVID-19 WBS. The RT-LAMP assay was developed for the detection of SARS-CoV-2 and its surrogate bacteriophage Phi6 by optimizing reaction conditions. The RT-LAMP assay can detect SARS-CoV-2 and Phi6 with limits of detection of 30 virions/ $\mu\text{L}$  and 10 gene copies/ $\mu\text{L}$ , respectively, in nuclease-free water. Grab sewage samples were collected from a nearby correctional facility experiencing outbreaks. Wastewater samples were subjected to RNA extraction after electronegative filtration for SARS-CoV-2 detection. High multiplexity of LAMPore was achieved by combinational DNA barcoding inserted into the forward inner primer of the RT-LAMP assay and use of Rapid Barcoding Kit for nanopore sequencing. Positive reads of the SARS-CoV-2 N region were identified by sequencing one pooled sample from ninety-six wastewater samples. De-multiplexing of the sequenced data was processed to assign ninety-six samples based upon the barcode sequences. The LAMPore assay results compare favorably with ddPCR assay results. The methods developed here will help to make WBS for COVID-19 and other public health threats more accessible.

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

### Sahar Abdelrazek

ID IGEP affiliated student Plant Pathology, Physiology and Weed Science

Faculty Mentor: Boris Vinatzer

### ***“Using metagenomics sequencing to investigate Xylella fastidiosa population and phylogeny within Virginia”***

Sahar Abdelrazek <sup>1\*</sup>, Marcela A. Johnson <sup>1</sup>, Parul Sharma <sup>1</sup>, Haijie Liu <sup>1</sup>, Elizabeth A. Bush <sup>2</sup>, Mizuho

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Pierce’s disease (PD) of grape caused by *Xylella fastidiosa* (Xf) subspecies *fastidiosa* (Xff) is a growing threat to the wine industry in Virginia (VA). PD severity is known to increase after warm winters and under drought conditions. With global warming and the expansion of the wine grape industry in VA, more VA regions, such as northern VA, that were never considered a high-risk area, have now become a high-risk region for PD. Metagenomic sequencing has been successfully used for fast detection and identification of plant pathogens. Long reads generated by the Oxford Nanopore Technologies (ONT) facilitate assembly of single-contig metagenome-assembled genomes (MAGs) and are shown to provide SNP resolution suitable for phylogenetic reconstruction for outbreak investigations. To inform management of Xf and associated outbreaks our main goal in this study is to investigate genetic and genomic characterization of Xf population present across VA using metagenomic sequencing. To achieve this goal, samples of grapevines, oaks, American sycamore, and hackberries with PD symptoms were collected and deep sequenced using ONT sequencing platforms. The obtained Xf reads were assembled into genomes for phylogenetic and comparative genomic analysis. Our results identified Xff to be associated with grapevines with PD symptoms while only Xfm was associated with the deciduous trees showing symptoms of leaf scorch. Metagenomic sequencing using ONT allowed us to obtain MAGs of sufficient quality for core genome analysis and whole genome based identification as long as the Xf Res-p gene qPCR Ct value was 20 °C or below. Phylogenetic analysis suggests the presence of two clonal lineages of Xff on grapevine and a genetically more diverse population of Xfm on oak and sycamore. The major challenge to obtain more MAGs of higher quality in this study was to increase read length and reduce host plant reads compared to Xf reads. Therefore, we will explore improvements to DNA extraction and sequencing in our future studies.



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

### **Paige Van de Vuurst**

ID IGEP affiliated student, TBMH

Faculty Mentor: Luis Escobar

### ***"Research equity in climate change and disease research"***

Authors: Paige Van de Vuurst<sup>1,2,3\*</sup> and Luis E. Escobar<sup>2,3,4,5</sup>

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\*Speaker

Climate change presents an imminent threat to almost all biological systems and is expected to amplify social inequity. Climate change is also expected to impact the spread and incidence of many diseases by augmenting host and vector ranges, contact rates among hosts, and disease emergence. In recent history there has been an increase in the number of empirical studies exploring how climate change has impacted infectious disease. The current diversity of topics, researchers, authorship equity, and geographic representation in climate change and infectious disease research is; however, unknown. To address these gaps of knowledge, we conducted a scoping study of climate change and disease research in scientific literature from 2015-2020. We found strong signals of research biases within the climate change and diseases field, with strong publications being skewed towards the disease transmission types and geographic areas studied. For example, vector-borne diseases associated with mosquitoes comprised the majority of publications. Geographic bias was also evident, with the bulk of studies being conducted in and authored by individuals affiliated with well-developed, high-income countries. The demographic trends identified by this study suggest an underrepresentation of minority groups in science. To aid in closing the gap of research inequity, we propose more diverse and inclusive practices on climate change and infection diseases research.

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

**Elizabeth Nowak**

Faculty

Department:

Faculty Mentor:

### ***""Histotripsy Treatment of Microbial Biofilms in Clinical Urinary Catheters"***

Title: Histotripsy Treatment of Microbial Biofilms in Clinical Urinary Catheters

Authors: Elizabeth Nowak<sup>a,b,c</sup>, Christopher Childers<sup>b</sup>, Ekta Bansala,<sup>b</sup> Ryan Morse<sup>b</sup>, Phyllis Whitehead<sup>b,d</sup>, Nammalwar Sriranganathan<sup>c,e</sup>, Eli Vlaisavljevich<sup>c,f</sup>, and Jayasimha Rao<sup>a,b,c</sup>

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Indwelling urinary catheters frequently develop intraluminal biofilms, increasing the risk for catheter-associated urinary tract infections (CAUTIs) and other systemic infections. Histotripsy is an image-guided and non-invasive focused ultrasound ablation method that has recently been shown to eliminate biofilms in-vitro from catheter surrogates. In this study, we present an investigation of histotripsy to treat biofilms in silicone urinary catheters collected from an inpatient unit between November 2021 and March 2022. Catheters were cut into equal segments and subjected to histotripsy with alternating segments serving as controls. Post-treatment, the intraluminal contents were assessed for cell viability and the segments were stained with crystal violet to quantify biofilm formation. Overall, three silicone catheters were collected with 12 segments in the treatment group and 12 corresponding controls. Results of tip cultures showed *Staphylococcus aureus*, a yeast, and mixed gram-negative rods with gram-positive organisms respectively. At the parameters used, there was an 81.7% reduction in biofilm after accounting for background uptake after a single scan of the catheter with histotripsy ( $p < 0.01$ ). Treatment did not appear to consistently eliminate cell viability after only a single histotripsy scan with pulse repetition frequency of 200Hz and estimated peak negative pressure of 31.6MPa. This is consistent with prior studies that showed higher treatment doses are required for a bactericidal effect. Additional studies are needed to optimize histotripsy treatment parameters and doses for achieving rapid biofilm removal and bactericidal effects and to assess the practicality of such treatment on indwelling catheters.

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

### **Greyson Moore**

ID IGEP affiliated student, Department of Biomedical and Veterinary Science

Faculty Mentor: Andrea Bertke

### ***"The Role of Sympathetic Neuronal Pathways in Regulating HSV1 and HSV2 Genital Infection"***

**G. A. MOORE<sup>1</sup>, S. LEE<sup>1</sup>, A. R. N. ABBOTT<sup>3</sup>, K. L. JOHNSON<sup>3</sup>, R. Wieske<sup>4</sup>, A. M. IVES<sup>1</sup>, A. S. BERTKE<sup>2</sup>**  
Biomedical and Veterinary Sciences<sup>1</sup>, Population Health Sciences<sup>2</sup>, Veterinary Medicine<sup>4</sup>,  
Virginia-Maryland College of Veterinary Medicine; Biological Sciences, Virginia Tech<sup>3</sup>

Herpes simplex virus (HSV) is one of the most common STIs and genital HSV lesions have been shown to increase risk three-fold the acquisition and spread of other STIs such as HIV. This painful, life-long disease is currently estimated to affect more than 85 million people in the US. HSV1 and HSV2 are closely related viruses with HSV1 typically associated with orofacial lesions and HSV2 associated with genital lesions, although these common clinical morphologies are not exclusive of each other. HSV establishes life-long infection by traveling retrograde along neuronal axons after primary infection to establish latency in sensory and autonomic neuronal cell bodies that directly innervate the genitourinary system. The latent virus can then become reactivated by a variety of stimuli, traveling back down the neuronal axons to induce a recurrence of painful lesions. Within the autonomic nervous system, HSV1 shows a preference for the sympathetic pathways whereas HSV2 shows a preference for the parasympathetic pathways suggesting that autonomic pathways may provide an alternative reservoir for reactivating virus that may contribute to different recurrence frequencies of HSV1 and HSV2. To determine the contribution of the sympathetic nervous system to acute genital disease and recurrence frequency, guinea pigs were treated with 6-hydroxydopamine (6-OHDA) prior to infection to ablate sympathetic neuronal axons, making them unavailable for infection and the establishment of latency. Chemical ablation of sympathetic axons significantly reduced severity and neurological involvement during acute disease for HSV1, but not HSV2 ( $p < 0.05$ ). Animals treated with 6-OHDA prior to infection also had reduced clinical recurrences by nearly 75% for HSV1 and by 50% for HSV2 ( $p \leq 0.001$ ). Thus, sympathetic neurons and pathways play a significant role in acute disease severity and neurological involvement of HSV1, but not HSV2. Additionally, sympathetic pathways are responsible for a significant portion of HSV1 and HSV2 recurrences, with a greater impact on HSV1 than HSV2.

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

### **Christina Chuong**

ID IGEP affiliated student, Department of Biomedical and Veterinary Science  
Faculty Mentor: James Weger-Lucarelli

#### ***"The Attenuation Mechanism of Interferon Gamma on Chikungunya Virus.***

Chikungunya virus (CHIKV) is an emerging pathogen that has caused millions of infections globally within the last 15 years and has the potential to become endemic in the US. CHIK disease is characterized by debilitating chronic arthritis which causes a significant reduction in quality of life. The current standard of care is based on treating symptoms and is often ineffective, and no vaccine is available to prevent disease. Furthermore, current CHIKV vaccine candidates in development lack key qualities of safety. This work aims to elucidate the mechanisms underlying the enhanced safety of a mouse interferon-gamma expressing CHIKV-Semliki Forest virus (SFV) chimera (CHIKV-SFV/IFN $\gamma$ ) vaccine. Previously, our group determined that mice vaccinated with the IFN $\gamma$ -expressing vaccine resulted in reduced footpad swelling and extremely limited viremia compared to the parental vaccine while offering full protection against wild-type CHIKV challenge. Towards understanding the mechanism by which this vaccine candidate is attenuated, we have identified three IFN $\gamma$ -regulated antiviral genes, GBP1, GBP2, and IDO1, that are highly upregulated in CHIKV-SFV/DomC-IFN $\gamma$  infected fibroblasts. We hypothesized that vaccine-driven IFN $\gamma$ -expression stimulates these genes to restrict viral replication in fibroblast cells. To scrutinize the role of these genes, we utilized siRNAs to knock down gene expression and infect with CHIKV-SFV/IFN $\gamma$  to see if attenuation is lost. Secondly, we also extended these studies to establish a human IFN $\gamma$  expressing vaccine to understand if these identified mechanisms could apply to human healthcare. This project will define a clear role for IFN $\gamma$  and its pathway activating GBP1/GBP2/IDO1 in modulating viral replication of CHIKV, particularly in regards to the enhancement of vaccine safety, and may identify novel strategies to improve vaccinations. Furthermore, the data obtained from these studies will establish a foundation to look into the long-term application of IFN $\gamma$  against pathogens threatening human health.

## **Breakout Session #5: Immunology and Host Pathogen Interactions**

### **Bryan Hsu**

Assistant Professor, Department of Biological Sciences

#### ***"Phages as commensal entities in the mammalia gut"***

Bacteriophages (phages) are among the most abundant entities in the human gut and can coexist with their cognate bacteria for long periods of time. Little is known about the functional impact of gut phages and how they influence the human host. Using mouse models to study the mammalian gut microbiota, we have found that virulent phages, i.e., those that infect bacteria, lyse them, and release phage progeny, can significantly alter commensal bacteria not directly targeted by phage and influence how the gut microbiota and host responds to antibiotics and diet. Our results suggest that naturally-resident gut phages are functionally important components of human health.

# Breakout Session #5: Immunology and Host Pathogen Interactions

## Kaylee Petraccione

ID IGEP affiliated student, Department of Biomedical and Veterinary Science

Faculty Mentor: Kylene Kehn-Hall

### ***Identification of LC3-interacting region (LIR) motifs within the NSs protein of Rift Valley fever virus as a method to discover host protein-viral protein interaction for therapeutic targeting.***

Kaylee Petraccione<sup>1,2</sup>, Mohamed Ali<sup>3</sup>, Nicole Bracci<sup>1,2</sup>, Normand Cyr<sup>3</sup>, Andrew Silberfarb<sup>4</sup>, Paul O'Maille<sup>5</sup>, James Omichinski<sup>3</sup>, Kylene Kehn-Hall<sup>1,2</sup>

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Rift Valley fever virus (RVFV) is a member of the family *Phlebovirus* and is a negative-sense RNA virus. RVFV is an arbovirus 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 airborne via virus-containing aerosols. Impregnated ruminant infections are characterized by abortion storms in which spontaneous abortion occurs in a large percentage of infected ruminants. In humans, the infection is typically mild, with symptoms such as headache, muscle pain, and fatigue. A small fraction of cases progress to a severe version of the disease that includes hemorrhagic fever or encephalitis. Continuing and unpredictable onslaughts by Rift Valley fever virus (RVFV) pose serious economic and health burdens worldwide, and 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 therapeutic target. We bioinformatically identified several LC3-Interacting Region (LIR) motifs within NSs, suggesting that NSs interacts with LC3 family members and influences autophagy. Autophagy is a homeostatic process in which cellular material is degraded and recycled and can be exploited by viruses to facilitate replication. Conversely, autophagy can be antiviral, which has been previously shown for RVFV. To determine whether NSs interacts with LC3-family proteins, plasmids encoding each of the six LC3-family members (LC3A, LC3B, LC3C, GABARAP, GABARAPL1 and GABARAPL2) were utilized. Interaction of NSs with all six human LC3-family members was observed through co-immunoprecipitation in virally infected cells. NSs also co-immunoprecipitated with endogenous LC3A and LC3B in virally infected cells. Mutation of NSs within one of the LIR motifs (NSs4) resulted in complete loss of LC3A binding as confirmed by co-immunoprecipitation. LC3 subfamily members (A-C) are acetylated on two conserved lysine residues (K49 and K51), and acetylated LC3 proteins are retained in the nucleus, thereby down-regulating autophagy. To determine the impact of acetylation of LC3 proteins on their interactions with NSs, plasmids expressing LC3A acetylation mimics were tested in cells. The LC3 acetylation mimics showed a significantly decreased interaction between NSs and LC3A, indicating that NSs preferentially interacts with de-acetylated LC3. To further explore the impact of NSs on autophagy, protein levels of the autophagy adapter protein p62/SQSTM1 were determined after RVFV infection. p62/SQSTM1 levels were significantly decreased in RVFV infected cells. In contrast, p62/SQSTM1 levels were partially rescued in MP-12 ΔNSs infected cells indicating that NSs contributes to the induction of autophagy. Ongoing studies are aimed at determining how NSs modulates autophagy and structurally characterizing critical regions of NSs required for the interaction with LC3 family proteins. Studying LIR motifs within viral proteins is an unexplored advancement in the identification of viral-host protein interactions, which is efficacious for novel therapeutic development. There is a high likelihood that new zoonotic viruses will emerge and/or current viruses will reemerge in new locations or cause significant outbreaks. Thus, our research to rapidly determine host factors and processes that can be therapeutically targeted to weaken the ability of these viruses to replicate is of critical importance to public health.

## Breakout Session #5: Immunology and Host Pathogen Interactions

### Lauren Panny

ID IGEP affiliated student, Department of Biomedical and Veterinary Science

Faculty Mentor: Kylene Kehn- Hall

### ***"Host interactors of the E1 glycoprotein of Venezuelan equine encephalitis virus as target for antiviral Drugs"***

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Venezuelan equine encephalitis virus (VEEV) is an emerging infectious disease that is transmitted by a mosquito vector. During the urban cycle VEEV is transmitted between equines that produce high viremia. In equine hosts, VEEV causes severe neurological complications and 50-70% of horses die from the infection. During this cycle, humans bit by infected mosquitos can become spillover casualties and are believed to be dead end hosts. Humans afflicted with the virus present with flu-like symptoms, malaise and fever with some cases resulting in encephalitis and less than 1% resulting in death. VEEV has been responsible for a number of epidemics throughout South and Central America and parts of Texas that have impacted both the agricultural and medical field. VEEV is classified as an alphavirus along with other emerging pathogens responsible for epidemics including, chikungunya virus (CHIKV), Sindbis virus (SINV) and eastern equine encephalitis virus (EEEV). Currently there are no FDA approved antiviral or vaccine options to combat alphaviruses. In order to expand potential antiviral targets, immunoprecipitation followed by LC-MS/MS was utilized to discover novel host interactors with E1, the fusion glycoprotein of VEEV. From our proteomic analysis we further explored the interactions of E1 with two different host interactors, protein disulfide isomerase family A member 6 and valosin-containing protein. Protein disulfide isomerases are proteins involved in the unfolded protein response (UPR) and are essential for disulfide bond formation and breakage in the ER and at the plasma membrane. Valosin-containing protein is involved in many cellular processes including autophagy, UPR and the ubiquitin proteasome system. Through our research we have shown two protein disulfide isomerase inhibitors, Loc14 and the FDA approved inhibitor nitazoxanide, effectively decrease VEEV titers in an in vitro model. Loc14 has also been shown to actively reduce a number of other emerging infectious diseases including EEEV, CHIKV, SINV, SARS-CoV-2, Rift Valley Fever virus and Zika virus. We have also found that the VCP inhibitor NMS-873 significantly reduces titers of VEEV. Current research aims to better understand the mechanisms of inhibiting these proteins and seeks to explore the effects of nitazoxanide against VEEV in an in vivo mouse model.

## **Breakout Session #5: Immunology and Host Pathogen Interactions**

### **Krisangel López**

ID IGEP affiliated student, Department of Entomology

Faculty Mentor: Jonathan Auguste

### ***“Development of animal models for the study of Cache Valley Virus”***

Authors: Krisangel López, John A. Muller, Sarah N. Wilson, Sheryl Countermash-Ott, Manette Tanelus, William B. Stone, Danielle L. Porier, Dawn I. Auguste, Orchid M. Allicock, Sally L. Paulson, Jesse H. Erasmus, Albert J. Auguste

Cache Valley Virus (CVV) is an emerging orthobunyavirus with significant importance to public health and the agricultural sector in North and Central America. CVV is associated with substantial agro-economic losses due to high embryonic lethality and developmental malformations in ruminants. CVV is also known to cause disease in humans, including fever, headaches, nausea, fatigue, encephalitis, meningitis, spontaneous abortions, and macrocephaly in infants. Although CVV pathogenesis has been well described in ruminants, small animal models are still unavailable, which limits the ability to study its pathogenesis and the development of new preclinical testing and therapeutics. Here, we have developed various murine, and avian models; including an immune-competent and -compromised murine models, to study CVV pathogenesis, tissue tropism and disease progression. Our results indicate that CVV disease in mice is dependent on innate immune responses and the type-I interferon signaling cascade to prevent infection. The IFNAR<sup>-/-</sup> mice infected with CVV developed lethal infections, with insignificant differences in age-dependent pathogenesis, which suggests this is the appropriate model for studying CVV both long and short term for in-depth pathogenesis studies and future therapeutic efforts. Additionally, we developed a novel CVV in utero model which demonstrated high levels of transmission, spontaneous abortions, and congenital malformations in fetuses. CVV infections presented with the highest tissue tropism in organs like the liver, spleen, and placenta. Our data show, that immune competent mice are generally unaffected by infection with CVV and only show disease in an age-dependent manner. Our avian models showed that immune competent birds do not develop significant viremia or disease when infected with CVV; and therefore, not an ideal model for studying this pathogen. Given CVVs broad dispersal throughout the Americas, high seropositivity rates among ruminants and humans, in addition to the geographic expansion of competent vectors, make the risk of emergence and exposure to CVV is incredibly high, and further interventions are needed to reduce risk of this important pathogen.



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

### **Anna Perez-Umphrey**

Postdoc, Department of Biological Sciences,  
Faculty Mentor: Dana Hawley

### ***"Sex and habitat drive hantavirus prevalence in marsh rice rat populations impacted by the Deepwater Horizon oil spill"***

Authors: Anna A. Pérez-Umphrey, Colleen B. Jonsson Andrea Bonisoli-Alquatic Allison M. Snider, Philip C. Stouffer, and Sabrina S. Taylor

*Bayou orthohantavirus* (BAYV) is one of several hantaviruses in the United States that cause hantavirus pulmonary syndrome in humans. Its host reservoir, the marsh rice rat (*Oryzomys palustris*), inhabits coastal saltmarshes of Louisiana, a region extensively impacted by anthropogenic disturbances, such as the 2010 Deepwater Horizon (DWH) oil spill. The oil spill presents an opportunity to investigate how a large-scale ecological disturbance can influence the hantavirus host–pathogen dynamic by examining BAYV presence in its reservoir host species in areas with different oiling histories. Here, we: (1) quantify BAYV prevalence in the rice rat in coastal saltmarshes of Louisiana; (2) assess whether prevalence is driven by rice rat demographics, seasonality, or association with habitat characteristics; and (3) determine whether these factors differ by marsh oiling history. We collected mark–recapture data and blood and tissue samples over 5 years (2013–2017) at oiled, unoiled, and reference sites. Testing of the samples for BAYV revealed an antibody and RNA prevalence of 13.7%. Logistic regression analysis found that prevalence varied seasonally and inter-annually, and in July of 2016 reached 30.8%. Sex (male) and increasing cover of *Sporobolus alterniflorus* and open water compared to *Juncus roemerianus* and bare ground were the strongest predictors of hantavirus prevalence. Abundance estimates derived from Huggins closed-capture models were greatest at oiled sites, but oiling treatment had no residual influence on BAYV prevalence, and abundance and prevalence were not correlated. This study supports the hypothesis that habitat is a main driver of hantavirus prevalence in the host and implies that continued and future disturbances in the region will likely impact the rice rat–BAYV dynamic by altering plant communities and landscape structure

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

### **Jiapeng Liu**

ID IGEP affiliated student, Department of Biochemistry

Faculty Mentor: Michael Klemba

### **"Enzymes responsible for the hydrolysis of fatty acids from exogenous lysophosphatidylcholine by asexual *Plasmodium falciparum*"**

Jiapeng Liu, Christie Dapper and Michael Klemba

Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA

Host lysophosphatidylcholine (LPC) is an important source of fatty acids for lipid synthesis by intraerythrocytic *Plasmodium falciparum*. Type A lysophospholipases are presumed to be responsible for liberation of free fatty acids from LPC. While numerous *P. falciparum* proteins have been annotated as lysophospholipases based solely on homology, the role and importance of each has not been comprehensively investigated. Taking a chemical biology approach, we developed an assay for in vivo LPC hydrolysis by parasitized erythrocytes and used it to screen a collection of inhibitors of mammalian lipases. These studies revealed that select inhibitors of enzymes of the serine hydrolase superfamily are able to block LPC hydrolysis. Using these inhibitors and a serine hydrolase-directed activity-based probe, we were then able to identify four candidate enzymes, two of which are exported to the erythrocyte. Next, we investigated the relevance of these enzymes for in vivo LPC hydrolysis by making a series of single, double, triple and quadruple knockout (KO) *P. falciparum* lines. Notably, the quadruple KO (QKO) line has lost the ability to efficiently hydrolyze LPC. Our findings support a model for LPC metabolism whereby it is hydrolyzed upon diffusion into the infected erythrocyte cytosol and the products are taken up by the parasite. We will report on the growth characteristics of QKO parasites in media with defined fatty acid sources (free fatty acids or LPC) and with human serum.

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

### **Karthikeyan Chandrasegaran**

Postdoc, Department of Biochemistry

Faculty Mentor: Clement Vinauger

### ***"Multi-threaded approach to understanding links between mosquito ecology and host-seeking behavior"***

Karthikeyan Chandrasegaran and Clément Vinauger

Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Mosquitoes are important vectors that claim about a million lives every year worldwide by transmitting a range of diseases. As larvae, they occupy diverse habitats and are influenced by many ecological factors that impact their adult life. Interestingly, the magnitude of these effects differs between males and females. Female mosquitoes show remarkable plasticity of body size in response to environmental variability. Also, body size in females strongly correlates with their adult behavior and reproductive traits. Here, we varied levels of intraspecific competition to quantify how larval conditions impacted olfactory responses of virgin and mated adult females seeking hosts for blood. The preliminary results suggest that host-seeking preferences are strongly linked to variations in female body size and mating status. Analysis of the head transcriptome of the large and small-sized females, both virgin and mated, reveals differences in genes linked to the onset of host-seeking and olfactory sensitivity. In my talk, I will discuss a novel multi-threaded approach that compares the gene transcripts' co-expression levels to identify 'hub genes' whose expression states likely mediate the links between larval ecology and adult host-seeking in mosquitoes. Using results from the transcriptomic analysis, we are pursuing electrophysiological investigations to understand the neural bases of the observed size and mating status-dependent variability in mosquito host-seeking behavior. These results will be discussed in the context of mosquito population dynamics and the ensuing disease consequences.

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

### **Alexandra Cumbie**

Postdoc, Department of Entomology

Faculty Mentor: Gillian Eastwood

#### ***"Seasonality of three human-biting ixodid ticks in western Virginia"***

In this study, we examine and report the phenological patterns of three medically important tick species in western Virginia, an understudied area in regards to ticks and tick-borne disease reports in the literature. Collections of *Ixodes scapularis* (the blacklegged tick), *Amblyomma americanum* (the lone star tick), and *Dermacentor variabilis* (the American dog tick) took place over a three year period (2019-2021) spanning across 6 counties. The results of this study give clarity for the active questing seasons for each of these species at the adult life stage; additionally these data provide insight into the associated risks of encountering an infected, human-biting tick in this region.

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

### **Paul Huxley**

Postdoctoral, Department of Statistics  
Faculty Mentor, Leah Johnson

### ***"Modelling the effects of environmental temperature on vectors of African trypanosomiasis"***

Paul Huxley and Leah Johnson (Department of Statistics)

Tsetse flies vector *Trypanosoma* parasites that cause sleeping sickness or African trypanosomiasis in humans and animals across sub-Saharan Africa. Sleeping sickness is an important source of mortality and morbidity in the region but so far, in the absence of effective vaccines, attempts to manage this burden have mainly focused on vector control technologies (e.g., the sterilised insect technique). A mechanistic trait-based understanding of how ectothermic disease vectors will respond to environmental change should enhance the effectiveness of vector control strategies and, ultimately, contribute to the goal of reducing the societal costs of the diseases they transmit. Using Bayesian approaches to fit thermal performance curves to tsetse fly life history data (i.e., survival, development, reproduction), we show how shifts in environmental temperature could affect the vector components of a dynamic transmission model framework for African trypanosomiasis.



Center for Emerging, Zoonotic,  
and Arthropod-borne Pathogens

# Abstracts

Poster Presentations



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## Antimicrobial Countermeasures (vaccine and drug)

### Ahmed Abouelkhair

ID IGEP affiliated student, Biomedical and Veterinary Science

Faculty Mentor: Mohamed Seleem

#### **Discovery of topical azoles as a promising therapeutic option for combating *Clostridioides difficile* infection**

*Clostridioides difficile* is an urgent public health threat and is a leading cause of healthcare-associated infection worldwide. Vancomycin and fidaxomicin are the only FDA-approved antibiotics for the treatment of *C. difficile* infection (CDI). However, CDI treatment is very challenging due to the increasing incidence of infections and the high treatment failure and recurrence associated with vancomycin and fidaxomicin treatment. Consequently, there is a critical need to discover new effective anti-*C. difficile* agents. Drug repurposing is a promising strategy to save the cost and time comparing to *de-novo synthesis*. Utilizing this strategy, we screened 3,200 FDA-approved drugs against *C. difficile*, and discovered miconazole, the topical azole antifungal, as a potent *C. difficile* inhibitor, with a minimum inhibitory concentration (MIC) of 1 µg/ml. Building upon this, we screened a library of 24 topical azoles against a wide panel of pathogenic *C. difficile* strains. Out of this library, 3 drugs exhibited the most potent activity against *C. difficile*, miconazole, econazole and tioconazole, which inhibited growth of 50% of tested isolates (MIC<sub>50</sub>) at concentrations of 1 µg/ml, 2 µg/ml and 2 µg/ml, respectively. In a time-kill assay, the 3 drugs were superior to vancomycin, reducing a high bacterial inoculum by more than 3 log<sub>10</sub> within 2-4 hours. The effects of increased *C. difficile* inoculum, and pre-exposure to simulated gastric intestinal fluid (SGF) and simulated intestinal fluid (SIF), on the activity of the azole drugs were also investigated. The drugs' antibacterial activity was stable in the presence of high bacterial inoculum, and they maintained their activity after being exposed to SGF and SIF. Furthermore, the 3 agents exhibited limited activity against key species that compose the host intestinal microbiota, including *Lactobacillus*, *Bifidobacterium longum*, and *Bifidobacterium breve*. Overall, this study provides topical antifungals that could be utilized for further development of potent and selective anticlostridial antibiotics.

## Antimicrobial Countermeasures (vaccine and drug)

### Nader Abutaleb

Postdoctoral Associate, Biomedical & Veterinary Science

Faculty Mentor: Xin Luo

### Old Drugs for Superbug: Repurposing Carbonic Anhydrase Inhibitors for Treatment of *Neisseria gonorrhoeae*

Nader S. Abutaleb, Ahmed E. M. Elhassanny, Alessio Nocentini, Chad S. Hewitt, Ahmed Elkashif, Bruce R. Cooper, Claudiu T. Supuran, Daniel P. Flaherty, and Mohamed N. Seleem

#### Abstract

*Neisseria gonorrhoeae* is an urgent public health threat worldwide that infects about 82.5 million patients annually. Due to the increasing incidence of infections that has been accompanied by an increase in the bacterial resistance to most antibiotics, the number of effective anti-gonorrheal therapeutic options is gradually diminishing. Currently, ceftriaxone is the only recommended antibiotic for treatment of *N. gonorrhoeae* infections. Yet, high-level gonococcal resistance to ceftriaxone is uprising. Without developing new anti-gonorrheal treatments, the world faces the real possibility of an untreatable gonococcal infection. Drug repurposing represents an attractive approach of drug discovery as it reduces the time, costs, and risks associated with traditional drug innovation. Utilizing this approach, we identified the FDA-approved carbonic anhydrase inhibitors (CAIs), acetazolamide (AZM) and ethoxzolamide (EZM), as potent anti-gonococcal agents. AZM and EZM displayed MIC<sub>50</sub>, against a panel of *N. gonorrhoeae* isolates, of 1 µg/mL, and 0.125 µg/mL, respectively. Both agents exhibited a bacteriostatic activity against *N. gonorrhoeae*, demonstrated post-antibiotic effects up to 10 hours, and no resistant mutants were isolated against both in the presence of a high bacterial inoculum. A permeability assay indicated that the increased anti-gonococcal potency of EZM *in vitro* is attributed to its increased permeability in *N. gonorrhoeae* as compared to that of AZM. Mechanistic investigations revealed that AZM and EZM inhibit *N. gonorrhoeae* carbonic anhydrase (CA). *N. gonorrhoeae* CA has been identified as an essential enzyme in *N. gonorrhoeae* that is required to maintain carbon dioxide and pH homeostasis. Both molecules exhibited almost similar potency against the gonococcal CA *in vitro*, where AZM displayed an inhibition constant ( $K_i$ ) of 74 nM, while EZM's  $K_i$  was estimated to 94 nM. Finally, the *in vivo* efficacy of AZM in a mouse model of *N. gonorrhoeae* genital tract infection was investigated. Compared to vehicle-treated mice, AZM significantly reduced the gonococcal burden by 90% in the vagina of infected mice after three days of treatment. Taken together, these results indicate that AZM and EZM warrant further investigation for translation into effective anti-*N. gonorrhoeae* agents to supplement the limited pipeline of anti-gonococcal therapeutics.

## Antimicrobial Countermeasures (vaccine and drug)

### Nour Alkashef

ID IGEP affiliated student

### Ritonavir as an effective adjuvant to amphotericin B for treating *Cryptococcus neoformans* infections

*Cryptococcus neoformans* causing life-threatening meningitis and represents an alarming global health threat associated with high mortality among immunocompromised individuals and HIV patients. The current arsenal of antifungal drugs to combat the growing problem of *cryptococcus* is very limited. Amphotericin B is the front line treatment for cryptococcal meningitis. However, the treatment with amphotericin B is commonly associated with severe adverse effects. In this study, we used the combinatorial approach to minimize the toxicity and to enhance the efficacy of amphotericin B against *C. neoformans*. We evaluate the HIV-protease inhibitor, ritonavir, as a potential co-drug to work synergistically and to enhance the effectiveness of amphotericin B treatment. Ritonavir exhibits potent in-vitro synergistic interaction when combined with amphotericin B against 100% (15/15) of the tested *C. neoformans* isolates with a fractional inhibitory concentration index ( $\Sigma$ FICI) ranging from 0.07 to 0.31. Notably, the combination of ritonavir with amphotericin B led to killing of all tested isolates within 3 hours as measured by time killing assays. As a part of the involved mechanistic study, ritonavir significantly interferes with glucose transport in *C. neoformans* reducing its uptake by 52%. These data highlight the potential of antifungal combination between amphotericin B and ritonavir to combat *C. neoformans* infections. Furthermore, these data will provide insight into the potential clinical usefulness of ritonavir because it is commonly administered in HIV-infected patients and cryptococcus is a leading cause of morbidity and mortality in those patients.

## Antimicrobial Countermeasures (vaccine and drug)

### Logan M. Breiner

ID IGEP affiliated student, Chemistry

Faculty Mentor: Andrew Lowell

### Structure-Activity Relationship of Epimerized and Non-epimerized Pleuromutilin Triazolyl Derivatives

Logan M. Breiner<sup>1</sup>, Roman Slowinski<sup>1,2</sup>, Andrew N. Lowell<sup>1</sup>.

<sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061.

Pleuromutilin derivatives have a long history of use as veterinary antibiotics, and recently as two FDA approved drugs. These drugs demonstrate slow resistance development, and while primarily active against Gram positive bacteria, they can be modified to gain Gram negative activity. In the present work, pleuromutilin has been azidated in two positions to enable convenient formation of 1,4-disubstituted triazoles using click chemistry. Click chemistry allows for the green and rapid generation of libraries of derivatives. Thirty-eight triazolyl derivatives have been generated, encompassing four class libraries. While the 22-triazolyl derivatives had activity comparable to pleuromutilins, the 20-triazolyls saw their activity abolished. Epimerization of the C12 position, followed by hydroazidation and triazole formation furnished the remaining two libraries. In preliminary antibiotic testing, these latter compounds all showed activity of  $\leq 8 \mu\text{g/mL}$  against *S. aureus*, with many showing the same level of activity against *E. coli*. Current work is focused on: 1. Generation of pleuromutilin/blasticidin S conjugates at the C22 position, and 2. MIC determination of the 12-*epi*-20-triazolylpleuromutilins.

## Antimicrobial Countermeasures (vaccine and drug)

### Nicolas Burns

ID IGEP affiliated student, Biomedical and Veterinary Science

Faculty Mentor: Mohamed Seleem

### Fungal Street Fighter: How combinational therapies can power up your frontline defenses

*Aspergillus fumigatus* responsible for more than 300,000 infections annually. Invasive pulmonary aspergillosis is a life-threatening fungal infection for immunosuppressed patients. Triazoles, including voriconazole, itraconazole, posaconazole, are the preferred agents for first-line therapy for immediate prophylaxis or treatment of infections due to *Aspergillus sp.*. Unfortunately, the growing number of azole-resistant strains of *A. fumigatus*, human and plant pathogenic species, has gained alarming attention due to treatment failure and high mortality. In recognition of this threat, the CDC has incorporated *A. fumigatus* to their watch list. Therefore, our group is searching for an adjuvant to re-sensitize azole-resistant *A. fumigatus* to the antifungal activity of current azoles. We identified the HIV-protease inhibitor, lopinavir (LPV), as a potent co-drug able to work synergistically with itraconazole and posaconazole against *A. fumigatus* isolates. Here, we investigated the interactions between LPV and different azole drugs (voriconazole, itraconazole, posaconazole) against multiple *A. fumigatus* clinically important isolates. Lopinavir displayed a synergistic relationship with itraconazole against 16 isolates ( $\Sigma$ FICI ranged from 0.188 to 0.375), while an indifference effect was observed against five isolates ( $\Sigma$ FICI ranged from 0.53 to 1.125). Remarkably, when lopinavir was combined with posaconazole, potent synergistic interactions were observed against 18 isolates ( $\Sigma$ FICI ranged from 0.091 to 0.188), while an indifference effect observed only against three azole-resistant isolates ( $\Sigma$ FICI ranged from 0.53 to 0.62). In addition, LPV was able to re-sensitize azole-resistant *A. fumigatus* strains, such as CDC 731 and CDC 733, to the antifungal activity of azoles. These results generate a new avenue of research and possible translatable clinical options that can re-sensitize or drastically reduce the azole burden needed to successfully treat *Aspergillus fumigatus* infections.

## Antimicrobial Countermeasures (vaccine and drug)

### Yehia Elgammal

ID IGEP affiliated student, Biomedical Sciences and Pathobiology

Faculty Mentor: Mohamed Seleem

### Evaluation of the HIV-protease inhibitor, atazanavir, for treatment of disseminated *Candida auris* infection

*Candida auris* represents an urgent public health threat worldwide that has been linked to numerous outbreaks around the world with a notably high mortality rate. Currently, only three classes of antifungal drugs (azoles, polyenes, and echinocandins) are available for the treatment of *C. auris*. The limited therapeutic options and the increased rate of drug resistance in *C. auris*, prompted us to evaluate a library of FDA-approved drugs for their ability to restore the antifungal activity of azole drugs. The screening identified the HIV-protease inhibitor, atazanavir, as a co-drug able to overcome azole resistance in *C. auris*. Atazanavir displayed potent *in vitro* synergistic interactions with itraconazole against 18/18 (100%) *C. auris* isolates with a fractional inhibitory concentration index ( $\Sigma$ FICI) ranging from 0.07 to 0.38. Remarkably, atazanavir restored the fungistatic activity of itraconazole against *C. auris* in an *in vitro* killing kinetics assay. Mechanistic studies revealed that atazanavir significantly interferes with *C. auris* efflux pumps which results in an increase of the Nile Red fluorescence by 50%. Additionally, atazanavir inhibits glucose transport and ATP synthesis causing the glucose utilization and ATP content in *C. auris* to decrease by 30% and 20%, respectively. The promising features of atazanavir/itraconazole combination prompted an evaluation of its *in vivo* efficacy in a mouse model of disseminated candidemia. Interestingly, this combination significantly reduced the burden of *C. auris* in mice kidneys, generating 1.15 log<sub>10</sub> CFU (~93%) reduction. Collectively, this study identifies atazanavir as a novel and potent azole chemo-sensitizing agent that merits further investigation.

# Antimicrobial Countermeasures (vaccine and drug)

## Cole Gannett

ID IGEP affiliated student

## Blasticidin S Derivatives: New Activity from an Old Drug

Cole Gannett<sup>1</sup>, Paige Banks<sup>1</sup>, Emily Mevers<sup>1</sup>, Andrew N. Lowell<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Virginia Tech, Blacksburg, VA (US)

The evolution of antimicrobial resistance (AMR) has introduced a very severe threat to human health and quality of life across the world. Unfortunately, the discovery of new antibiotics to help combat this emerging resistance has significantly slowed down in the past few decades. In order to stay in the fight against AMR, new strategies are clearly needed to develop useful antibiotics. One such strategy is the repurposing of underexplored antimicrobials that were discovered but never developed as antibiotics. Blasticidin S is one of these forgotten antimicrobials. It is a ribosome-binding antimicrobial with activity against both Gram-negative and Gram-positive pathogenic bacteria as well as eukaryotes. Very few semi-synthetic derivatives of this compound exist but one has shown increased potency against bacteria and decreased potency against eukaryotic organisms. We created a library of eight ester derivatives of Blasticidin S and screened them against a number of Gram-negative and Gram-positive pathogens and one representative eukaryotic fungal pathogen. The smaller esters retained the important Gram-negative activity of blasticidin S while increasing the activity against Gram-positives. Interestingly, none of the ester derivatives were active against the fungal pathogen while Blasticidin S was. These results confirm that semi-synthetic derivatization of these compounds has the potential to increase potency against bacteria and decrease the toxicity to the eukaryotic ribosome, paving the way for development into clinically relevant antibiotics.



# Antimicrobial Countermeasures (vaccine and drug)

## Anna Hassebroek

ID IGEP affiliated student, Biomedical and Veterinary Science

Faculty Mentor: X.J. Meng

## Vaccination of K18-hACE2 transgenic mice with a hepatitis B core antigen-based virus-like particle vaccine expressing SARS-CoV-2 T/B cell epitopes induces epitope-specific humoral and a cell-mediated immune responses

Author list: Anna M. Hassebroek<sup>1</sup>, Harini Sooryanarain<sup>1</sup>, C. Lynn Heffron<sup>1</sup>, Seth A. Hawks<sup>1</sup>, Tanya LeRoith<sup>1</sup>, Thomas E. Cecere<sup>1</sup>, William B. Stone<sup>2</sup>, Debra Walter<sup>3</sup>, Hassan M. Mahsoub<sup>1</sup>, Bo Wang<sup>1</sup>, Debin Tian<sup>1</sup>, Hannah M. Ivester<sup>1</sup>, Irving C. Allen<sup>1</sup>, A. Jonathan Auguste<sup>2</sup>, Nisha K. Duggal<sup>1</sup>, Chenming Zhang<sup>3</sup>, Xiang-Jin Meng<sup>1</sup>

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The hepatitis B core antigen (HBcAg) contains three different sites that tolerate insertion of foreign immunogenic epitopes. After epitope insertion, the protein self-assembles into a virus-like particle (VLP) and can be used as potential vaccine candidate. In this study, a HBcAg-based VLP expressing SARS-CoV-2 T-cell and B-cell epitopes was produced and tested for its immunogenicity and protection against SARS-CoV-2 in K18-hACE2 transgenic mice. The SARS-CoV-2 Spike protein epitopes used in this study were identified *in silico* and are predicted to stimulate both humoral and cell-mediated immune responses. The vaccine and control (HBcAg with no epitope insertion) constructs were cloned into a pET-28a expression plasmid and transformed into BL21 (DE3) *E. Coli* cells. After induction of the transformed *E. Coli* for target protein expression, the vaccine and control proteins underwent a two-step purification process that resulted in a highly purified product of both constructs. The VLP formation of the vaccine candidate and control protein was observed via transmissible electron microscope. K18-hACE2 transgenic C57BL/6 mice were vaccinated intramuscularly and boosted twice with either vaccine or control VLP. Mice were challenged with SARS-CoV-2 virus at three weeks following the final booster. Mice were monitored for up to eight days after challenge and evaluated for humoral and cell-mediated immune responses, clinical disease, and viral RNA load in the lung. A subset of mice was vaccinated but not challenged, and necropsied one week after the second booster dose to evaluate humoral and cell-mediated immune responses prior to virus challenge. The vaccinated mice in this subset showed a significant increase in epitope-specific IgG levels compared to baseline, had more memory CD8<sup>+</sup> T-cells by flow cytometry, and higher IL-6 and MCP-1 expressions by cytokine bead assay. While not statistically significant, there was also evidence of a Th1 response in some vaccinated mice, with high levels of IFN- $\gamma$  and TNF production. These results indicate the HBcAG-based SARS-CoV-2 VLP vaccine can elicit both humoral and cell-mediated immune responses. We are in the process of evaluating whether the observed immune responses induced by the VLP vaccine can confer any protection against SARS-CoV-2 infection in K18-hACE2 mice.

## **Antimicrobial Countermeasures (vaccine and drug)**

### **Kathryn Hayes**

ID IGEP affiliated student, Translational Biology, Medicine and Health

Faculty Mentor: Brandon Jutras

### **Drug screen to determine efficacy of different $\beta$ -lactams against *T. pallidum* in vitro**

Authors: Kathryn A. Hayes, Julianne M. Dressler, Brandon L. Jutras

Syphilis is a multiphasic, chronic, sexually transmitted disease which poses a global health threat. Since its discovery, Penicillin has been the primary treatment option for syphilis. Its efficacy against the causal pathogen, *Treponema pallidum*, is significantly greater compared to doxycycline, the only other viable treatment option thus far. Due to previous inability to culture this pathogen in vitro, little work has been done to identify compounds that rival the efficacy of penicillin, and the laborious nature of the culturing process has limited recent drug studies to one to a few compounds. With growing concern of antibiotic resistance as well as global penicillin allergies and shortages, alternative treatment options are needed. In this study, almost 100  $\beta$ -lactams were screened using phases of successive biological replicates to efficiently determine efficacy in vitro. In addition to manual bacterial enumeration, multiple qRT-PCR targets validated the efficacy of the top performing compounds. In follow up studies we determined the minimum inhibitory concentration of top performing compounds. Our efforts identified six  $\beta$ -lactams with comparable efficacy to penicillin. All of these compounds had interpolated MIC values of about one nanogram per milliliter or less. This is the first major drug screen to identify new therapeutics effective against *T. pallidum* growth in vitro. Our findings also provide potential candidates for further clinical investigation.

# Antimicrobial Countermeasures (vaccine and drug)

## Casey Hensley

ID IGEP affiliated student, Biomedical and Veterinary Science

Faculty Mentor: Lijuan Yuan

### **Combined live oral priming and intramuscular boosting regimen with Rotarix and a nanoparticle-based multivalent rotavirus vaccine confers significant protection against both G4P[6] and G1P[8] human rotavirus infection in gnotobiotic pigs**

Casey Hensley<sup>1</sup>, Charlotte Nyblade<sup>1</sup>, Peng Zhou<sup>1</sup>, Viviana Parreno<sup>1</sup>, Annie Frazier<sup>1</sup>, Maggie Frazier<sup>1</sup>, Ariana Fantasia-Davis<sup>1</sup>, Sarah Garrison<sup>1</sup>, Ruiqing Cai, Ming Xia<sup>2</sup>, Ming Tan<sup>2</sup>, Lijuan Yuan<sup>1</sup>

<sup>1</sup> Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24060, USA; <sup>2</sup> Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

Human rotavirus (HRV) is the causative agent of severe dehydrating diarrhea in children under the age of five, resulting in up to 215,000 deaths each year. With the introduction of live, oral attenuated vaccines nearly two decades ago, these deaths almost exclusively occur in low and middle-income countries where vaccine efficacy is the lowest. Reasons for decreased efficacy in these areas include gut dysbiosis, concurrent use of other live oral vaccines, concurrent enteric viral infection and malnutrition. These factors make parenteral vaccines for HRV particularly attractive, as they bypass the GI tract and thus avoid many of the issues associated with live oral vaccines. Our collaborators at Cincinnati Children's Hospital Medical Center developed a nanoparticle-based nonreplicating, parenteral HRV vaccine, utilizing the shell (S) domain of the capsid of norovirus (NoV). The S domain of NoV self-assembles into a nanoparticle with exposed C-termini, which are amenable to the insertion of foreign proteins. In this vaccine, HRV's VP8\* from the three most predominant P types, P[4], P[6], and P[8] were fused to the exposed C-termini, forming the S<sub>60</sub>-VP8\* cocktail nanoparticle vaccine. The safety, immunogenicity and protective efficacy of this cocktail vaccine was evaluated using gnotobiotic (Gn) pig models of P[6] and P[8] HRV infection and diarrhea. We also evaluated a prime/boost strategy, using one dose of the commercial oral Rotarix vaccine, followed by one dose of the intramuscular (IM) cocktail nanoparticle vaccine. A group of pigs receiving only the S<sub>60</sub> nanoparticle with no fusion of HRV VP8\* was used as the control. Gn pigs who received the Rotarix/cocktail nanoparticle regimen, but not two doses of the cocktail nanoparticle alone, had significantly shortened duration of virus shedding when challenged orally with the virulent Wa (G1P[8]) HRV. Furthermore, Gn pigs who received this combined prime/boost regimen had a significantly shortened mean duration of virus shedding, along with a significantly reduced AUC of virus shedding after challenge with Arg (G4P[6]) HRV. Rotarix/cocktail-vaccinated pigs challenged with P[8] HRV had significantly higher P[8]-specific IgG antibody secreting cells (ASCs) in the spleen post-challenge. Rotarix/cocktail-vaccinated pigs challenged with P[6] HRV had significantly higher numbers of P[8]-specific IgA ASCs in the spleen post-challenge, as well as significantly higher numbers of P[6] and P[8]-specific IgG ASCs in the ileum post-challenge. Pigs vaccinated IM with the cocktail nanoparticle vaccine and challenged with P[8] HRV had significantly higher numbers of P[4] and P[8]-specific IgG ASCs in the ileum post-challenge. Pigs vaccinated with the cocktail nanoparticle vaccine and challenged with P[6] HRV had significantly higher numbers of P[8] and P[6]-specific IgG ASCs in the spleen, as well as significantly higher numbers of P[8]-specific IgG ASCs in the ileum post-challenge. Thus, the cocktail nanoparticle IM vaccine alone primed for significant virus-specific B cell immune responses, but failed to confer significant protection. These results suggest the promise and warrant further investigation into the oral priming and parenteral boosting strategy for future HRV vaccines.

# Antimicrobial Countermeasures (vaccine and drug)

## Abdullahi Jamiu

ID IGEP cohort, Biomedical and Veterinary Sciences

Faculty Mentor: Kylene Kehn-Hall

## Development of novel synthetic rocaglates with antiviral activity against Venezuelan equine encephalitis virus

Abdullahi Jamiu<sup>1,2</sup>, John Porco<sup>3,4</sup>, Lauren Brown<sup>3,4</sup>, Kylene Kehn-Hall<sup>1,2</sup>

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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne positive sense single-stranded RNA virus that belongs to the genus Alphavirus within the family Togaviridae. VEEV causes disease in both equines and humans. In humans, the symptoms of VEE include moderate flu-like symptoms, including fever, headache, nausea, and fatigue, which can progress to severe neurological complications, including seizures, coma, confusion, and photophobia, in up to 14% cases, resulting in an associated mortality of 1%. Moreover, 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 the USDA. There are currently no FDA-approved therapeutics or licensed vaccines against VEE in humans. Recently, the development of inhibitors targeting cellular mechanisms essential for viral replication and proliferation has gained increased traction because such inhibitors will reduce the risk for antiviral resistance development and might potentially demonstrate broad-spectrum 'pan-antiviral' activity. Generally, viruses, including Alphaviruses, depend on cellular translation machinery for viral protein synthesis. The eukaryotic translation initiation factor 4A (eIF4A) has been identified as an essential factor for viral protein synthesis. Rocaglates are a class of natural compounds that inhibit eIF4A-dependent translation initiation. These compounds have been shown to exhibit antiviral activity against some RNA viruses, including Zika virus, hepatitis E virus, and SARS-CoV-2. However, no study has reported the effects of any natural or synthetic rocaglates against New World Alphaviruses, including VEEV. This study involved evaluating the antiviral activity of synthetic rocaglates against VEEV. Eight (33.3%) out of the twenty-four tested synthetic rocaglates exhibited inhibitory activity at nanomolar concentrations against VEEV TC83 NanoLuc reporter virus with 50% effective concentration (EC<sub>50</sub>) values ranging from 21.75 nM to 149.4 nM. Two of these compounds, BUCMD00584 and BUCMD00589, displayed impressive activity with EC<sub>50</sub> of 21.75 nM and 35.54 nM, respectively. These compounds were also well tolerated by Vero cells with CC<sub>50</sub> value greater than 10,000 nM and an estimated selective index value greater than 280. Furthermore, in silico studies also showed that the most experimentally active compounds, BUCMD00584 and BUCMD00589, interact and bind more strongly with human eIF4A (PDB: 5ZC9) compared to the reference inhibitor, rocaglamide A (-6.91 kcal/mol) and an experimentally inactive rocaglate, BUCMD00543 (-3.642 kcal/mol), with docking scores of -8.113 and -8.107 kcal/mol, respectively. Future studies will involve elucidating the structure-activity relationship of these two top hits and determining the inhibitory effects of these compounds on VEEV virion production, RNA levels, translation, and protein levels. The antiviral activity of these compounds against other New-World Alphaviruses and Old-World Alphaviruses will be also evaluated.

# Antimicrobial Countermeasures (vaccine and drug)

## Babatomiwa Kikiowo

ID IGEP affiliated student

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### **Construction of ceftriaxone-resistant and azithromycin-resistant *Neisseria gonorrhoeae* strains suitable for a mouse study**

Babatomiwa Kikiowo, Aloka B. Bandara, Nader S. Abutaleb and Mohamed N. Seleem

Gonorrhoea is a sexually transmitted infection caused by *Neisseria gonorrhoeae*, a human pathogen that can colonize male and female genital mucosal surfaces. Gonorrhoeal infections pose a significant global public health concern; the Centers for Disease Control and Prevention reported 677,769 cases of gonorrhoea in 2020. The rapid occurrence of *N. gonorrhoeae* resistance to all classes of antibiotics could lead to untreatable gonorrhoea. Thus, the development of novel anti- *N. gonorrhoeae* drugs is urgently needed. *N. gonorrhoeae* strain FA1090 is the only strain used for in vivo mouse model because of its natural resistance to streptomycin, a necessary antibiotic utilized in the models to inhibit the commensal flora in the lower genital tract of mice to enhance *N. gonorrhoeae* colonization. However, the strain is susceptible to all antibiotics used to treat *Neisseria*, such as ceftriaxone and azithromycin, and not suitable for drug discovery. To test the efficacy of new drugs against clinical isolates, such as ceftriaxone-resistance and azithromycin-resistance strains in vivo, the streptomycin resistance is a required phenotype for performing the in vivo mouse model. Hence, our aim in this study is to construct streptomycin-resistant mutants of two clinically important *N. gonorrhoeae* strains exhibiting resistance to the frontline antibiotics used in the treatment of gonorrhoea, ceftriaxone and azithromycin that could be utilized in vivo. The *rpsL* gene encoding for resistance to streptomycin was isolated from *N. gonorrhoeae* FA1090 and cloned into the pMR32 plasmid. The resultant plasmid was introduced into wild-type *N. gonorrhoeae* WHO-X (ceftriaxone-resistant) and CDC-181 (azithromycin-resistant) strains. The resulting mutant strains were confirmed to be resistant to streptomycin (minimum inhibitory concentrations, MICs >1024 µg/mL). A pilot study to determine the infectious dose of the newly developed mutant strains of WHO-X and CDC-181 was performed using a murine model of *N. gonorrhoeae* genital tract infection. Groups (n=5) of ovariectomized female 8-week-old BALB/c mice were implanted with a 5-mg, 21-day controlled-release estradiol pellet subcutaneously and were inoculated intravaginally with 20 µL of different doses of *N. gonorrhoeae*. An antibiotic cocktail was administered throughout the experiment to increase the susceptibility to *N. gonorrhoeae* and minimize vaginal flora. Vaginal swabs were collected every other day and *N. gonorrhoeae* CFU were counted. The constructed streptomycin-resistant mutants, like the *N. gonorrhoeae* FA1090 strain, survived in mice for seven days. Based on this study, the newly constructed streptomycin-resistant strains have the potential to be used in mouse models to investigate the efficacy of drugs against azithromycin-resistant and ceftriaxone-resistant *N. gonorrhoeae* strains.

# Antimicrobial Countermeasures (vaccine and drug)

## Zachary Kohanov

ID IGEP affiliated student, Chemistry

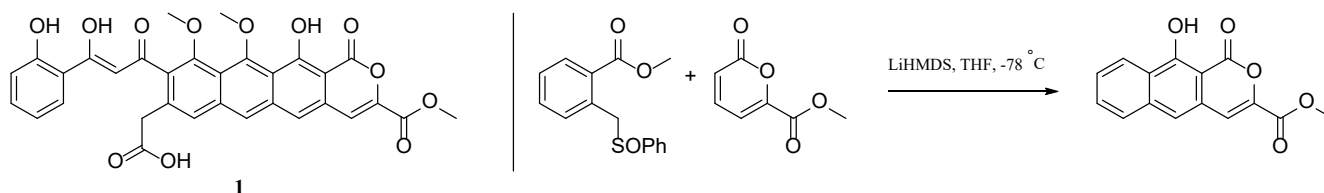
Faculty Mentor: Andrew Lowell

## Optimization of the Hauser Annulation with Unsaturated Lactones: Total Synthesis of Thermorubin

Zachary A. Kohanov<sup>1</sup>, Suzzudul I. Shuvo<sup>1</sup>, Andrew N. Lowell<sup>1</sup>

<sup>1</sup>Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA

Due to the increasing rise of antibacterial resistance, more potent antibiotics with novel mechanisms of action are required to kill life-threatening bacterial infections. An understudied molecule Thermorubin (1) represents such a molecule: with binding sites on both the 30S and 50S ribosomal units, thermorubin binds to the full 70S ribosomal structure and inhibits protein translation, preventing the creation of more bacterial proteins. This bacteriostatic mechanism of action is unique to this naphthoisochromenone, but due to its difficult isolation, poor oral-bioavailability, and relative instability when exposed to air it has been overlooked as an effective way to combat bacteria. A total synthesis towards the creation of thermorubin has been proposed that will incorporate the use of sulfoxide intermediates to create the tetracyclic core of thermorubin to allow for creation and derivatization of the antibiotic to increase water solubility, as well as create a more potent molecule to fight life-threatening infections.



## **Antimicrobial Countermeasures (vaccine and drug)**

### **Lois Kwane Kyei**

ID IGEP affiliated student, Chemistry

Faculty Mentor: Emily Mevers

### **Screening Microbial Extracts from Moon Snail Egg Masses for Biofilm Inhibition**

Lois K. Kyei, Emily Mevers

Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA

Bacterial biofilms are multicellular communities of bacteria that adhere to surfaces and are enclosed in a matrix of extracellular polymeric substances. Biofilms provide extra protection to bacteria and are shown to be 10-1000 times more resistant to antibiotics than their planktonic counterparts. Bacterial biofilms have led to an influx in the rise of antibiotic resistance and represent a substantial setback to current antimicrobial therapeutics. In the human body, biofilms colonize medical devices and develop in immunocompromised individuals, and cause persistent infections that are difficult to treat. Biofilms are difficult to eradicate and when medical devices cause the infection, the only treatment is removal of the device, causing huge financial constraint on patients. The immense detrimental effects of bacterial biofilms have prompted our effort to develop molecules that inhibit their formation or eradicate already formed biofilms. In my research, I am employing extracts from microbial symbionts of moon snail eggs to identify novel metabolites with biofilm inhibiting abilities. The moon snail egg mass and its microbial symbionts represent a unique ecological niche that have been understudied. These microbial symbionts have been hypothesized to produce small molecules that chemically defend the eggs. Isolating the biologically relevant compounds could lead to the discovery of potent novel biofilm-inhibiting compounds. Thus far, I have screened our library of crude extracts against two biofilm-forming bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Extracts inhibiting at least 50% of the biofilm growth were considered active and are being prioritized for further chemical evaluation in the medically relevant biofilm-eradication assay. Bioassay-guided isolations and structure elucidations is an on-going effort.

# Antimicrobial Countermeasures (vaccine and drug)

## Hsin-Wen Liang

ID IGEP affiliated student

### Novel antibiotics against *Neisseria gonorrhoeae* accelerated by drug repurposing

Hsin-Wen Liang<sup>1</sup>, Ahmed E.M. Elhassanny<sup>1</sup>, Nader S. Abutaleb<sup>1</sup>, and Mohamed N. Seleem<sup>1</sup>

<sup>1</sup> Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA

*Neisseria gonorrhoeae*, the second most common bacterial cause of sexually transmitted infections (STIs), is listed as an urgent-threat pathogen by the Centers for Disease Control and Prevention (CDC). Due to the growing prevalence of resistance development against first-line treatment and several classes of antibiotics, the discovery of new anti-gonorrhea therapeutics is an urgent need. Drug repurposing significantly reduces the time and expense of traditional drug development. In this study, we utilized a drug repurposing approach and screened 3,802 FDA-approved and clinical drugs against *Neisseria gonorrhoeae* FA1090. A total of nine novel non-antibiotics compounds were identified in the screening with significant anti-gonococcal activity. The activity of these compounds was tested against multidrug-resistant *N. gonorrhoeae* clinical isolates. The minimal inhibitory concentrations (MICs) of these nine compounds are ranging from 0.03 to 8 µg/ml. One of the drugs, anti-inflammatory drug auranofin, that showed potent activity *in vitro* (0.06 µg/ml) was investigated *in vivo* against *N. gonorrhoeae* using a female murine model of vaginal infection. Mice treated with auranofin at a dose of 0.25 mg/kg showed a 1.04 (91%) and 1.40 (96%) log<sub>10</sub> reduction after three and five days respectively comparing to the vehicle. In conclusion, drug repurposing is a productive approach for drug discovery. The findings from this study provide the foundation for future gonorrhea therapeutics research.



## **Antimicrobial Countermeasures (vaccine and drug)**

### **Austin Lowry**

ID IGEP affiliated student, Chemistry

Faculty Mentor: Andrew Lowell

### **Creating New Chloramphenicol Derivatives Via a Semi-Synthetic Approach**

Austin C. Lowry, Andrew N. Lowell

Department of Chemistry at Virginia Tech

To fight against pathogenic bacteria that have developed resistance to various antibiotics, developing new derivatives from existing scaffolds provides an avenue to next generation antibiotics that are effective against these resistant pathogens. One antibiotic of interest for derivatization is the broad-spectrum antibiotic chloramphenicol. Chloramphenicol has become a drug of last resort due to its toxicity but synthetic derivatives have shown to be just as effective and safer alternative. A new method has been developed to synthesize new chloramphenicol derivatives by bypassing several synthetic steps. The aromatic nitro group would be reduced down to an amine which would then be used to generate various amides and sulfonyl groups. This approach creates new derivatives in the para position on the aromatic ring without affecting any of the chlorine and alcohol groups that are present.

## Antimicrobial Countermeasures (vaccine and drug)

**Rusha Pal**

ID IGEP affiliated student

### **Antisense inhibition of RNA polymerase $\alpha$ subunit of *Clostridioides difficile***

*Clostridioides difficile*, the etiology of antibiotic-associated diarrhea and pseudomembranous colitis, has emerged as a major enteric pathogen in recent years. Antibiotic treatment perturbs the gut microbiome homeostasis, facilitating the colonization and the proliferation of the pathogen in the host intestine. Paradoxically, the clinical repertoire for *C. difficile* infection includes antibiotics vancomycin and/or fidaxomicin. The current therapies do not address the perturbed gut microbiome, which supports recurrence of infection after cessation of antibiotic therapy. Peptide nucleic acids (PNAs) are a class of nucleic acid analogues are novel alternatives to traditional antimicrobial therapy. PNA are capable of forming strong and stable complexes with RNA and DNA thus permitting targeted inhibition of specific genes. Here, we report a novel PNA that can target RNA polymerase  $\alpha$  subunit (*rpoA*) in the multi subunit bacterial RNA polymerase (RNAP) responsible for transcribing DNA to RNA in *C. difficile*. Based on the genome sequence of *C. difficile* 630, we designed anti-*rpoA* constructs conjugated to 5 different cell penetrating peptides (CPP). The most promising PNA conjugate (*rpoA*-TAT) was further evaluated for its efficacy *in vitro*. The designed anti-*rpoA* construct could inhibit clinical isolates of *C. difficile* at clinically relevant concentrations (MIC values ranging between 4 to 8  $\mu$ M) and exhibited bactericidal activity superior to the standard-of-care antibiotics. Interestingly, the efficacy of the designed PNA conjugate remained unaffected even when tested at different pH and against a high inoculum of the pathogen. The *rpoA*-TAT was very specific against *C. difficile* and did not inhibit members of the gut microflora. Furthermore, silencing of the *rpoA* gene suppressed the expression of genes encoding for *C. difficile* virulence factors [toxin A (*tcdA*), toxin B (*tcdB*), and master regulator of sporulation (*spoOA*)]. Taken together, our study confirms that *rpoA* gene can be a promising narrow-spectrum therapeutic alternative for tackling CDI.

## Antimicrobial Countermeasures (vaccine and drug)

### Ehab Salama

ID IGEP affiliated student, Biomedical Sciences and Pathobiology

Faculty Mentor: Mohamed Seleem

### Lansoprazole potentiates the antifungal activity of amphotericin B against multi-drug resistant *Candida auris*, targeting the cytochrome bc1 complex

*Candida auris* has emerged as a problematic fungal pathogen associated with high morbidities and mortalities. Amphotericin B is the most effective and broad-spectrum antifungal agent used for treatment of invasive fungal candidiasis with extremely rare resistance among clinical isolates. However, the clinical efficacy of this drug has been impacted recently with the emergence of *C. auris* which possessed extraordinary resistant profile against all available antifungal drugs, including amphotericin B. There is an urgent need for novel antifungal agents or co-drugs capable of restoring/enhancing the antifungal activity of amphotericin B and reducing its toxicity. In this study, by screening a panel of ~3,400 FDA-approved drugs we identified the proton pump inhibitor, lansoprazole, as a potent enhancer for the activity of amphotericin B against *C. auris*. Lansoprazole exhibited potent synergistic interactions with amphotericin B against 18/20 (90%) *C. auris* isolates with  $\Sigma$ FICI ranged from 0.25 to 0.5. Proteome Integral Solubility Alteration (PISA) assay revealed that lansoprazole inhibits an essential target in the yeast cytochrome system (Rieske protein of the mitochondrial cytochrome bc1 complex) leading to increase in the oxidative stress in the fungal cells which consequently augment the oxidative damaging effect of amphotericin B on *C. auris* cells. The target was confirmed with the rotenone rescue assay and transcriptome sequencing (RNA-Seq) analysis. Most importantly, lansoprazole restored the *in vivo* efficacy of amphotericin B in an immunocompromised mouse model, resulting in a 1.7-log (~98%) CFU reduction in the kidney burden of *C. auris*. In conclusion, our results identified lansoprazole as a potent enhancer to the antifungal activity of amphotericin B in addition to identification of mitochondrial cytochrome bc1 as a novel drug target to overcome the antifungal resistance in *C. auris*.

# Antimicrobial Countermeasures (vaccine and drug)

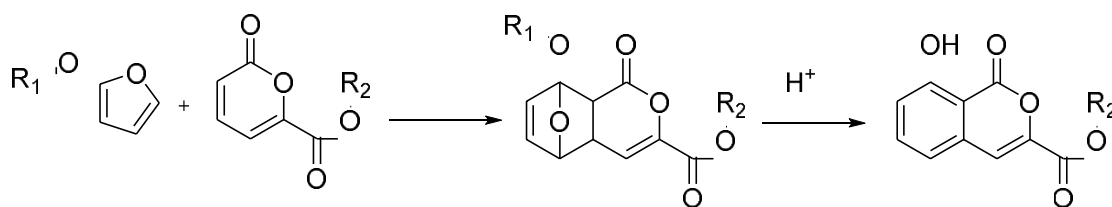
## Suzzudul Islam Shuvo

ID IGEP affiliated student, Chemistry

Faculty Mentor: Andrew Lowell

## Using Total-synthesis And Semi-synthesis To Find Novel Natural Products Derivatives

Isocoumarin rings are a common moiety in natural products, which is why chemists have been interested to synthesize the moiety efficiently. We propose a novel total-synthetic route to generate isocoumarin rings starting from the various ester or amide derivatives of 2-pyrone-6-carboxylic acid. These derivatives can be generated by a simple 3 step process<sup>1,2</sup>. We are looking at 2(5H)-Furanone as a four-carbon unit that can be added to the pyrone ring to synthesize the phenol portion of the isocoumarin.



R<sub>1</sub>= Protecting group

This technology will also unlock a potential synthetic route to make highly substituted phenols. After the isocoumarin products have been synthesized, their activities will be tested against different microorganisms and further synthetic modifications will be performed to access for more active compounds. Synthesis is a powerful tool which we are using to create novel Tetracycline derivatives as well. By using electrophilic aromatic substitution the C-7 and C-9 positions<sup>3</sup> of tetracycline can be modified and by using Mannich conditions<sup>4</sup> the 2-amido position of tetracycline will be derivatized. After these derivatives have been tested for activity, the potential for attaching a linker in these positions will be explored. Using linkers will activate the potential of the tetracycline derivatives with improved activity, to connect with a suitable antibiotic attaching to a site near tetracycline in the bacterial ribosome. The resulting 'bidentate' antibiotics will be further assessed for activity against resistant microorganisms.

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# Antimicrobial Countermeasures (vaccine and drug)

## Samantha Tollefson

ID IGEP affiliated student, Chemistry

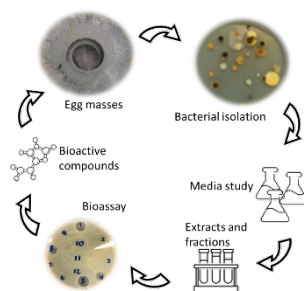
Faculty Mentor: Emily Mevers

## Antimicrobial discovery from bacteria associated with Puerto Rican moon snail eggs

Samantha Tollefson\*, Erik Payne\*, Carla Menegatti and Emily Mevers

*Department of Chemistry, Virginia Tech, Blacksburg, VA*

Moon snails are marine organisms that deposit their egg masses without subsequent parental care. The egg masses are rich in nutrients; however, they are not attacked by any other organism. This suggests the use of a chemical protection mechanism to defend themselves. 1 2 Symbiotic bacteria could be responsible for producing bioactive metabolites able to protect the egg masses against pathogens and predators. 1 The lack of studies on marine egg masses led us to investigate the chemical potential of their microbiota. Samples from the moon snail eggs were collected in Puerto Rico. As of now, 51 bacterial strains have been isolated. DNA extraction and 16S rRNA sequencing are being carried out in combination with chemical studies. Bacteria were cultivated in three different media to elicit the production of different metabolites. Extracts were obtained, fractionated, and tested against various pathogens to prioritize bioactive compounds for isolation. Dereplication through LC-MS and NMR allowed the identification of two analogues peptides described as A-3302-A and B and nocardamine, a cyclic peptide. 3 4 There is ongoing work to complete the workflow discussed to ensure each bacteria is properly tested.



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# Antimicrobial Countermeasures (vaccine and drug)

## Wei Wang

ID IGEP affiliated student, Civil & Environmental Engineering

Faculty Mentor: Peter Vikesland

## Surface enhanced Raman spectroscopy of bacterial metabolites to unveil bacterial tolerance to antibiotics

*Wei Wang, and Peter J. Vikesland \**

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The presence of antibiotics in waterbodies can facilitate the development and proliferation of antimicrobial resistance (AMR) - one of the greatest public health threats currently facing humankind. An improved understanding of AMR is of great societal importance. In this study, we report the use of surface enhanced Raman spectroscopy (SERS) for the monitoring the bioactive metabolites of two ampicillin resistant *Pseudomonas aeruginosa* strains and the identification of bacterial antibiotic resistance mechanisms. The time-dependent SERS results show that multiple bioactive metabolites can be determined during bacterial growth and the blue-green pigment pyocyanin (PYO) dominates. PYO accumulates during the early growth stage and is subsequently consumed or diffuses into the culture medium. In the presence of ampicillin at concentrations below the minimal inhibitory concentration (MIC), *P. aeruginosa* growth is maintained at a rate consistent with the control. The nutrient consumption and the production of most of the metabolites are not affected. However, the SERS signal of PYO is strongly promoted. PYO acts as a quorum sensing signaling molecule and the increase of PYO concentration can promote the transcription of antibiotic resistance genes. We further detected the metabolic SERS signals of ampicillin susceptible *Escherichia coli* and found that exogenously added PYO promotes *E. coli* growth, even in the presence of ampicillin. The results indicate that PYO confers antibiotic tolerance not only for the producing species, but also other cocultured bacterial strains. Our work provides new techniques and insights to better understand bacterial antibiotic tolerance mechanisms and has implications for effectively addressing the threat of AMR.

# Computational Biology and Disease Modeling

## Anthony Krueger

ID IGEP affiliated student, Mathematics

Faculty Mentor: Lauren Childs

### **Assessing the practical identifiability of a malaria model to evaluate the validity of conversion rates**

*Plasmodium falciparum* is the most virulent malaria species that affects humans. During its lifecycle, the parasite exists in two forms, asexual and sexual. Within the human host, the asexual parasites primarily replicate in the bloodstream. During the replication cycle, some asexual parasites commit to produce sexual parasites, which cannot reproduce in the human host. The sexual parasites continue the infection cycle after being ingested by a mosquito during a blood meal. The proportion of asexual parasites committed to forming sexual parasites in a given replication cycle is known as the “conversion rate.” The evaluation of the conversion rate is paramount to the understanding of the transmission and therefore control of *Plasmodium falciparum*. In 2000, Diebner et al. created a collection of deterministic models for the sexual parasitemia determined by conversion rate, asexual parasitemia, and mortality. Continuing this work in 2001, Eichner et al. determined a range of conversion rates through fitting their chosen best model from Diebner et al. to over 100 individual patient infections. The average conversion rate generated from this work is often cited in modelling studies. Here, we evaluate the practical identifiability of parameters for the chosen best model and simplifications of this model. Focusing on a small collection of well-documented patients, we fit all parameters of the model, including the conversion rate, and obtain predicted sexual parasitemia. Using these predicted trajectories as a baseline truth, we simulate a collection of sexual parasitemia trajectories with varying levels of noise. We fit all parameters for each simulated data set in the collection and then evaluate the measure of accuracy to our original parameters to determine if we have practical identifiability. We find that we do not have practical identifiability of parameters, except for two patients with the shortest time span for the simplest model. Without parameter identifiability, the reported conversion rates may need to be reconsidered.

# Computational Biology and Disease Modeling

## Zachary Spencer

ID IGEP affiliated student, Population Health Sciences Department

Faculty Mentor: Nick Ruktanonchai

## Quantifying change in mobility and corresponding risk of COVID-19 and future emerging disease spread in counties across Virginia

Zachary Spencer, Kayla Vaught, and Nick Ruktanonchai

Since the emergence of COVID-19 in late 2019, public health agencies have had to deal with numerous waves of COVID-19. These waves are strongly linked to mobility patterns in various communities, which can change rapidly depending on sociocultural context. In the United States, events such as university openings led to localized waves of COVID-19, likely because they involve large numbers of potentially infected students travelling to places with colleges and universities. Conversely, cases typically decrease when mobility is reduced, such as when shelter-in-place orders were in place, which also prevents the spread of COVID-19 cases to new areas.

Understanding how changes in mobility manifested across the COVID-19 pandemic is essential for predicting and controlling future waves of not only COVID-19, but also predicting how future emerging diseases may spread. By knowing where, when, and why mobility increases and how long different communities adhere to travel restrictions, we can better predict where future outbreaks may occur and how they may spread.

Here, we quantified travel patterns across Virginia throughout the COVID-19 pandemic, using a SafeGraph human mobility dataset obtained from mobile phone users statewide. We found that the duration and intensity of reduced mobility varied greatly between counties after the initial lockdowns in March 2020. Urbanicity was a primary driver of these patterns, as mobility returned to normal in some rural counties during summer 2020, but generally remained low in metropolitan areas. Across all months, metropolitan counties experienced greater reductions in mobility than rural counties. Metropolitan counties also experienced greater reductions for longer periods than rural counties, as rural counties returned more closely to baseline levels of mobility by February 2021. We also found that counties with universities and colleges over 5000 students experienced large increases in local travel in September 2020 that did not occur elsewhere. Our results provide context for the higher rates of COVID-19 generally observed in rural areas throughout the pandemic, and in areas with universities when universities reopened, suggesting that they are strongly related to increases in human mobility patterns which lead to increased contact and exposure. These analyses will help inform key high-risk events and locations when future infectious diseases emerge, and demonstrate that human mobility data can be used to identify these high-risk events and locations.



# Ecology, Epidemiology & Environmental Microbiology

**Md Rasheduzzaman**

Postdoc

## **Controlling Legionella in Building Water Systems: Role of Water Temperature and Disinfectants**

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*Legionella* spp. occurring in building water systems are causing serious pneumonic infections worldwide including in the United States. Thermal treatments and chemical disinfection (i.e., adding chlorine and monochloramine) are among the key strategies to control their occurrence in large building water systems. Although there are existing guidance documents and regulations on using hot water temperature and appropriate disinfectant residual levels to control *Legionella*, a lack of consensus requires more evidence-based research. We performed the first systematic review and meta-analyses of the available literature evidence on the association between water temperature and *Legionella* contamination to identify the required water temperature for controlling *Legionella*. Another meta-analysis is currently underway to identify the effective disinfectant and corresponding residual concentration for controlling the risk of *Legionella* contamination. For identifying the required water temperature, thirteen studies met our inclusion criteria while screening is still ongoing for disinfectants. The odds ratio (OR) for detecting *Legionella* at temperatures >55°C compared to lower temperatures from a meta-analysis of three studies was 0.17 [95% CI: 0.11, 0.25], which indicates a strong negative association between temperature and *Legionella* colonization. A logistic regression on results from multiple studies found a temperature of 58°C associated with a 10% probability of detectable *Legionella*. Our qualitative synthesis and meta-analysis found that the required hot water temperature should be maintained between 55°C to 58°C to avoid detectable *Legionella* contamination in hotel hot water systems if other control factors are also in place such as proper flow balancing, removal of dead legs, etc. Our preliminary qualitative synthesis for disinfectants revealed that chloramine was effective to reduce *Legionella* contamination in both the hot- and cold-water samples compared to chlorine and a disinfectant chlorine residual level of ≥0.2 mg/L was an effective level of concentration to control *Legionella* colonization. Required hot water temperature and the effective disinfectant levels identified in this study may be useful in planning and policymaking to regulate *Legionella* spp. contamination in building water distribution systems.

# Ecology, Epidemiology & Environmental Microbiology

## Landon Bilyeu

ID IGEP affiliated student, School of Plant and Environmental Sciences

Faculty Mentor: David Schmale

Little is known about the transport and fate of aerosolized particles associated with harmful algal blooms (HABs). An Airborne DRone Particle-monitoring System (AirDROPS) was developed and used to monitor, collect, and characterize airborne particles over two HABs in Grand Lake St Marys (GLSM) and Lake Erie (LE), Ohio USA in August 2019. The AirDROPS consisted of an impinging device (ID) and an optical particle counter (OPC) mounted on a large commercial quadcopter (DJI Inspire 2). The sensor package was mounted above the airframe to limit the effects of propeller downwash that can corrupt measurements taken below the drone. Twenty flights were conducted 10 m above water level (AWL) at GLSM, and five flights were conducted 10 m AWL at LE. This sampling height was chosen to minimize the effects of propwash on aeroolization from the lake surface. One intercomparison flight was conducted at GLSM over land adjacent to a sonic anemometer mounted on the top of a flagpole 15 m above ground level (AGL). Particle counts generally decreased from morning to afternoon flights, ranging from highs above 4000 in the morning to below 1000 later in the day. Decreased particle counts were associated with an increase in windspeed that corresponded with time of day, ranging from highs above 4000 below 4 m/s to below 2500 above 4 m/s. Flow cytometry was used to image particles trapped in a liquid impinger onboard the AirDROPS. Sixty percent (15/25) of the impinge samples contained at least one biotic (fluorescent) object. Impinger samples were also analyzed for a suite of potential cyanotoxins using liquid chromatography–mass spectrometry (LC-MS/MS), but no cyanotoxins were detected in any of these air samples (water samples collected during a similar time contained greater than 20 parts per billion microcystins). Additional work is needed to understand the environmental factors associated with the potential aerosolization and transport of cyanobacterial cells and toxins in aquatic environments.

# Ecology, Epidemiology & Environmental Microbiology

## Auja Bywater

ID IGEP affiliated student, Food Science and Technology

Faculty Mentor: Monica Ponder

### The survival of *Campylobacter* sp. and *Escherichia coli* O157:H7 inoculated onto kale during refrigerated storage

**Authors:** Auja Bywater, Kathleen Alexander, Joseph Eifert, Laura K. Strawn, and Monica A. Ponder

**Introduction:** *Campylobacter* and pathogenic *Escherichia coli* illnesses are attributed to consumption of fresh produce, especially lettuce and other leafy greens. While the survival of human pathogens has been documented on many salad greens, little attention has been paid to kale. Kale is increasingly consumed raw and may be contaminated through pre or post-harvest activities. Due to the extended shelf life of kale, it is warranted to examine the survival of *Campylobacter* sp. and pathogenic *E. coli* O157:H7 inoculated onto kale stored in a controlled environment at 4°C, ± 2°C, and average humidity of 95% over a 23-day period.

**Methods:** The following strains were used: *C. Coli* ATCC 35223, *C. jejuni* ATCC 33291, *E. coli* (ETEC) H10407 serotype O127:H11(NR-4), *E. coli* (EPEC) JPN15 serotype O127:H6(NR-50517), *E. coli* EHEC strains O157:H7 ATCC 43894, and *E. coli* O157:H7 ATCC 43895. Kale was collected from a local producer and sorted to remove broken and damaged leaves. Spot inoculation was used to deposit 100ul across the kale leaf surface (25g) in 10ul drops. The kale was dried then placed in plastic bags and stored at 4°C, ± 2°C for 23 days. The kale leaves were destructively sampled on days 0 (after drying), 1, 2, 3, 5, 7, 9, 11,13, 15, 17, 19, 21, and 23. Experimental design included three replicates per microorganism and time point. For each timepoint, kale inoculated with the individual pathogen cocktails from each of the replicates (n=3 for each microbe per time point) and one bag of non-inoculated kale were sampled. Bacteria were removed from leaf surfaces by using a combination of hand massage and lab blending in sterile 0.1% peptone. Diluent was serially diluted 10-fold in 0.1% sterile peptone and plated onto Tryptic Soy Agar to enumerate total heterotrophic bacteria and the appropriate selective and differential media (CCDA for *Campylobacter* and EMB for *E. coli*). The non-inoculated sample was serially diluted in 0.1% sterile peptone and plated on all three media types. Enrichment was performed for 1ml of the initial dilution to increase sensitivity of detection of *Campylobacter* (Bolton's Broth) and *E. coli* (Brilliant Green Bile Broth 2%) and screened by PCR. All plates and enrichments were held at 37°C for 24h. On the CCDA, only small, flat, and gray colonies were counted as *Campylobacter*. On the EMB, only colonies with a green sheen were counted as *E. coli*. All colony types were counted on the TSA. The limit of detection for this method (considering enrichment and PCR) is 10 CFU. From day 5 and forward, 1.5ml of the enrichment from the *Campylobacter* and *E. coli* samples were extracted and screened with developed PCR protocols.

**Results:** *E. coli* log CFU/g on the inoculated kale were 4.8 Log CFU (SD = 0.41) at day 0 and declined over time (linear regression,  $p < 7.72e-08$ ,  $R^2 = 0.43$ ). *E. coli* could be detected by serial dilution and plating on EMB until day 13 and by enrichment and PCR until day 19. *Campylobacter* recovered from day 0 inoculated kale was 1.8 Log CFU/g (SD = 0.76) and decreased significantly over time (linear regression,  $p < 1.92e-06$ ,  $R^2 = 0.32$ ). *Campylobacter* remained detectable by serial dilution and plating on CCDA through day 7 and until day 13 when enrichment and PCR were used. During refrigerated storage the population of heterotrophic aerobic bacteria increased by 1.2 log CFU/g (SD = 0.4).

**Significance:** The microbial load of *Campylobacter* and *E. coli* on fresh kale reduced over time in a temperature and humidity-controlled environment; however, these foodborne pathogens remained detectable through the majority of period when kale was still acceptable to consumers.

**Acknowledgement:** This research was supported through NSF award number 2009717

**Keywords:** shelf-life, food-borne pathogens, produce, leafy greens

# Ecology, Epidemiology & Environmental Microbiology

## Shreya Choudhary

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: David Popham

### Characterizing the role of a family of lipoproteins in *Bacillus subtilis* spores

Germination of *B. subtilis* spores is mediated by a series of stages. It begins when germinants bind to the Ger receptors at the inner spore membrane, leading to cascading events controlled by various protein complexes. One important event in this process is cortex hydrolysis, which is mediated by Germination-Specific Lytic Enzymes (GSLEs), SleB and CwlJ. Previous studies have identified a family of highly abundant spore lipoprotein paralogs associated with the inner spore membrane, including Ylaj, YhcN, YutC, and CoxA. It was shown that these lipoproteins play a role in efficient spore germination. Studies in our lab show that the lipoproteins interact with each other, as well as with SleB and its partner protein YpeB.

We speculate that these lipoproteins form a complex that stabilizes the germination apparatus. This could be either directly through protein-protein interactions or indirectly through membrane ordering, resulting in efficient spore germination. Our goal is to understand the complex formed by these lipoproteins. To examine lipoprotein localization within the spore, we will be using dual-colored Photo-Activated Localization Microscopy (PALM). To further understand lipoprotein interactions, we will use tools like Isothermal Titration Calorimetry (ITC) to examine the affinity of interaction. Bioinformatics-based approaches like EVcouplings and molecular docking will be used to predict the precise interacting amino acid partners. To determine if the lipoproteins produce a protected environment for other germination-active proteins, we will examine protein accessibility using a chemical biotinylation approach. Studying the germination apparatus assembly would further our understanding about the process of germination and in turn help develop efficient methods of spore killing.

# Ecology, Epidemiology & Environmental Microbiology

## Deeya Deeya

ID IGEP affiliated student, Chemistry

Faculty Mentor: Emily Mevers

### Identifying Defensive Metabolites from Freshwater Sponge and its Symbionts

Marine sponges are known to produce bioactive compounds that have the potential to combat infections and are host to many microbial symbionts. Many of these pharmaceutically relevant metabolites that were originally isolated from the sponge are now known to be produced by their microbial symbionts, both cyanobacteria and bacteria. Interestingly, very little research has been conducted on the secondary metabolites of freshwater sponges, although they are known to have symbiotic partners like the marine sponges. This inspired our current research effort into the defensive small molecules produced by freshwater sponges and their symbionts (i.e., algae and bacteria). *Ephydatia muelleri* is a freshwater sponge that is found in rivers along the Eastern US and it is known to harbor *Chlorella*-like endosymbiotic microalgae, in addition to a complex bacterial community. We hypothesize that the algae and bacterial symbionts are likely to produce defensive secondary metabolites to protect its sessile animal host against predation and pathogens. Preliminary chemical investigations into freshwater sponges from Richmond, Virginia, and their symbiotic algae has led to refined chemical fractions possessing antimicrobial activity. Further purification of the metabolites in the extract of the algae has led to a metabolite that exhibited modest activity against both gram-positive and gram-negative bacteria and fungal pathogens. Work is ongoing to identify the active components.

# Ecology, Epidemiology & Environmental Microbiology

## Roxanna Farzad

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Frank Aylward

## Genomics and Environmental Distribution of Giant Viruses in the North Pacific Subtropical Gyre

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<sup>2</sup> Center for Emerging, Zoonotic, and Arthropod-Borne Infectious Disease, VirginiaTech, Blacksburg VA, 24061

Large double-stranded DNA viruses from the phylum *Nucleocytoviricota* are ubiquitous members of marine ecosystems that are important agents of mortality for eukaryotic plankton. These “giant viruses” include isolates with the largest genomes (up to 2.5 Mbp) and physical dimensions (up to 1.5  $\mu\text{m}$ ) of any viruses discovered to date. Although giant viruses are known to be prevalent in marine systems, their activities in oligotrophic ocean waters remain unclear. Oligotrophic gyres constitute the majority of the ocean and assessing viral activities in these regions is therefore critical for understanding overall microbial processes in the ocean. In this study, we generated 11 metagenome-assembled genomes (MAGs) of giant viruses from samples previously collected from Station ALOHA in the North Pacific Subtropical Gyre. We performed phylogenetic analyses that revealed they belong to the orders *Imitervirales* (n=6), *Algavirales* (n=4), and *Pimascovirales* (n=1). Genome sizes ranged from 120-574 kbp, and several of the genomes encoded predicted TCA cycle components, cytoskeletal proteins, collagen, rhodopsins, and proteins potentially involved in other cellular processes. Comparison with other marine metagenomes revealed that several have broad distribution across ocean basins and represent abundant viral community members in pelagic surface waters. Our work sheds light on abundant giant viruses present in oligotrophic ocean waters across the globe.

# Ecology, Epidemiology & Environmental Microbiology

## Regina Hanlon

Research Associate, School of Plant and Environmental Sciences

Faculty Mentor: David Schmale

## Drone-based water sampling and characterization of three freshwater harmful algal blooms in the United States

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Keywords: Harmful algal bloom, HAB, drone, freshwater lake, UAS, cyanobacteria, toxin, phycocyanin, nutrients

Freshwater harmful algal blooms (HABs), caused mostly by toxic cyanobacteria, produce a range of cyanotoxins that threaten the health of humans and domestic animals. Climate conditions and anthropogenic influences such as agricultural run-off can alter the onset and intensity of HABs. Little is known about the distribution and spread of freshwater HABs. Current sampling protocols in some lakes involve teams of researchers that collect samples by hand from a boat and/or from the shoreline. These collections are often restricted to certain months of the year, and generally are only performed at a limited number of collection sites. In lakes with active HABs, surface samples are generally sufficient for HAB water quality assessments. We used a unique DrOne Water Sampling SystEm (DOWSE) to collect water samples from the surface of three different HABs in Ohio (Grand Lake St Marys, GLSM and Lake Erie) and Virginia (Lake Anna), United States in 2019. The DOWSE consisted of a 3D-printed sampling device tethered to a drone (uncrewed aerial system, or UAS), and was used to collect surface water samples at different distances (10–100 m) from the shore or from an anchored boat. One hundred and eighty water samples (40 at GLSM, 20 at Lake Erie, and 120 at Lake Anna) were collected and analyzed from 18 drone flights. Our methods included testing for cyanotoxins, phycocyanin, and nutrients from surface water samples. Drones offer a rapid, targeted collection of water samples from virtually anywhere on a lake with an active HAB without the need for a boat which can disturb the surrounding water. Drones are, however, limited in their ability to operate during inclement weather such as rain and heavy winds. Collectively, our results highlight numerous opportunities for drone-based water sampling technologies to track, predict, and respond to HABs in the future.

# **Ecology, Epidemiology & Environmental Microbiology**

## **Sangita Karki**

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Frank Aylward

## **Prevalence of Gene Transfer from Giant Virus to Eukaryotes**

Sangita Karki and Frank Aylward

Department of Biological Sciences

Giant viruses are capable of lytic infection of the eukaryotic host and are considered “pickpockets” of the genes and play a major role in horizontal gene transfer. It is said that the horizontal gene transfer mainly occurs from cell to virus and virus to virus and that the transfer of a gene from virus to the host is rare. The recent advancement of bioinformatics tools and the availability of eukaryotic and viral sequences have helped surge the discovery of Endogenized Viral Elements (EVEs) and have raised the speculation on the direction of transfer of genes between the host and the viruses. The endogenization capacity of retroviruses and eukaryotic giant viruses made the reassessment of study of transfer of genes between host and viruses possible and provide some evidence of the transfer of genes from virus to host. Nevertheless, there is still a large void in the evidence of the transfer of genes from virus to the host. Eukaryotic signature proteins (ESPs) are proteins that are found in greater than 95 percent of the eukaryotic genomes. In our study, we implement bioinformatics tools to find ESPs, assess the functional category of these proteins and their distribution in giant viruses, and reconstruct phylogenetic tree to know how prevalent the transfer of the gene from virus to host is.



# Ecology, Epidemiology & Environmental Microbiology

## Sydney Kinstler

ID IGEP affiliated student

### **A microbial physiology approach to the detection and enumeration of *Clostridium perfringens* in the poultry house environment**

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Necrotic enteritis (NE) is a significant intestinal disorder of poultry caused by the spore-forming, obligate anaerobe, *Clostridium perfringens*. Antimicrobials have historically prevented NE until concerns over antibiotic resistance resulted in pressures to limit use in poultry, causing NE reemergence. *C. perfringens* spores persist in the environment, including poultry farms and potentially cause significant disease. Our central hypothesis is avian pathogenic *C. perfringens* strains produce high spore loads on some farms, predisposing birds to NE by spore ingestion from a heavily contaminated environment. Litter samples were collected in areas suspected of high spore loads in houses with varying histories of NE with suspicions of an outbreak. Capitalizing on *C. perfringens* physiology and metabolism, we have developed a media formulation that “poisons” other poultry environmental bacteria using an oxidizing agent coupled with a physical heat treatment to determine spore abundance. The medium was formulated to be selective and differential for *C. perfringens* spores compared to other species in poultry litter that cannot survive conditions where nitrate reductase activity produces cytotoxic chlorite from a chlorate source, such as *C. perfringens* can. Lecithinase activity by *C. perfringens* is visible on egg yolk, a lecithin source that forms distinct white zones around the colonies. Coupled with a heat treatment to eliminate vegetative cell growth, spore isolation from poultry litter was achieved. Using our medium formulation and heat treatment, we identified lecithinase-positive colonies from the poultry house environment and subsequently confirmed them as *C. perfringens* by MALDI-TOF. We specifically found *C. perfringens* spores in the environment of poultry farms with a history of NE compared to farm without NE. The ability to monitor *C. perfringens* spore abundance is a game changer to understanding NE on poultry farms.

# Ecology, Epidemiology & Environmental Microbiology

## Brian Jacob Kohler

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: David Popham

## Analysis of *Clostridioides difficile* CwID Amidase Activity on *Bacillus subtilis* Cortex Peptidoglycan

*Clostridioides difficile* is a highly antibiotic resistant and infectious endospore forming bacterium. The formation of an endospore is necessary for the survival of the bacterium while in-transit between hosts and while passing through the toxic environment of the host's stomach. Essential to the endospore's resistance is a thick layer of highly modified peptidoglycan called the cortex. While the endospore cortex is forming, the enzymes CwID and PdaA convert N-acetylmuramic acid (NAM) into Muramic- $\delta$ -lactam (MAL). MAL serves as a recognition element for germination specific lytic enzymes that degrade cortex peptidoglycan layers during germination. Without the MAL residues the endospore cannot complete germination. Unique to the Peptostreptococcaceae family is the lipoprotein GerS, which is required for the function of CwID. The interaction between these two proteins is poorly understood. Attempts to complement this system in *Bacillus subtilis* have been unsuccessful. No MAL residues were found while testing cortex peptidoglycan and none of the mutants produced are capable of germinating. The next phase of this study will focus on the *in vitro* binding and activity of *C. difficile* CwID on vegetative peptidoglycan with or without the presence of GerS. This will determine if GerS is needed for the localization of CwID within the forespore and/or if it is necessary for the enzymatic activity of CwID. Furthering the understanding of *C. difficile*'s germination machinery will potentially provide new targets for therapies.

# Ecology, Epidemiology & Environmental Microbiology

## Nichole Laggan

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Joseph Hoyt

## Host abundance and heterogeneity in infectiousness determine extent of the environmental reservoir

### Authors:

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Environmental pathogen reservoirs exist in many globally important disease systems and can fuel disease outbreaks, affect pathogen evolution, and increase the threat of extinction. Differences in pathogen shedding among hosts can create a mosaic of infection risk on the landscape by influencing the establishment of the environmental reservoir and increasing pathogen contamination in high use areas. However, the establishment of the environmental reservoir in multi-host communities and how factors like host infection and abundance influence pathogen contamination remains an important outstanding question. Here we examine the environmental reservoir of *Pseudogymnoascus destructans*, the pathogen that causes white-nose syndrome in bats, by combining data on pathogen shedding, infection intensities, host abundance, and the subsequent propagule pressure imposed by each species within the community. We observe interspecific heterogeneity in pathogen shedding of *Pseudogymnoascus destructans* among bat species and find that host infectiousness and abundance have the greatest effect on the extent of pathogen in the environmental reservoir. The contribution to the environmental reservoir by each species varied dynamically, and interactions between abundance, infection prevalence and intensity moderated the effect of the propagule pressure imposed by each species. Ultimately our results show that multiple factors, which vary among species and over time, interact to determine the extent of the environmental reservoir.

# Ecology, Epidemiology & Environmental Microbiology

## John Maurer

Professor, Animal and Poultry Science  
CALs

## Static Incubation and Anaerobic Digestion Reduce Class 1 Integrons and Associated Antimicrobial Resistance Genes in Poultry Litter

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There is significant concern that agricultural use of antimicrobials leads to spill over of antimicrobial resistance (AMR) into the general population. Animal manures contain a large and diverse reservoir of AMR genes. While poultry litter is a rich source of nitrogen, it contains an abundance of AMR genes and genetic elements associated with its dissemination. Reduce this reservoir, and its potential to transmit AMR to pathogens is diminished. Four litter amendments: intermittent wet-dry, aerobic, anaerobic, and static incubations were assessed for their impact on the community resistome. A litter sample was taken prior to the treatments and served as a control. Bacteria were extracted from the litter and separated from detritus using spin columns. DNA was extracted from the bacteria and normalized to 10 ng/ $\mu$ L. qPCR was used to estimate the gene load of streptomycin-resistance, sulfonamide-resistance, and class 1 integron-integrase genes *aadA*, *sul1*, and *int11* respectively in the poultry litter community. A standard curve was created using *E. coli* pDU202 to estimate gene copy numbers in the samples. The initial litter sample contained an average of 10.76 log<sub>10</sub> bacterial genomes per gram. The wet-dry, aerobic, and anaerobic treatments significantly reduced the genomes per gram of litter by  $\sim$  0.6 log<sub>10</sub>. The anaerobic incubation of litter reduced the bacterial genomes per gram the most, from 10.76 to 10.11 log<sub>10</sub>. All treatments significantly reduced levels of *sul1* in the litter from 9.45 log<sub>10</sub> to 8.53-8.87 log<sub>10</sub> *sul1* per gram litter. *aadA* was significantly reduced by the anaerobic incubation by 0.52 log<sub>10</sub> and increased with the aerobic incubation by 0.41 log<sub>10</sub>. While levels of *int11* per gram litter were reduced in each treatment, none of the treatments had a statistically significant impact on gene load (p-values: 0.03 to 0.21). When levels of *aadA* were normalized to total bacterial genomes' following each treatment, the wet-dry and aerobic incubations significantly increased *aadA* per bacterial genome by 0.81 and 1.03 log<sub>10</sub> respectively. The aerobic and anaerobic treatments significantly increased the amount of *int11* per bacterial genome by 0.45 and 0.31 log<sub>10</sub> respectively. *sul1* per bacterial genome was significantly reduced by the static treatment by 0.63 log<sub>10</sub> (p = 0.0006). The anaerobic incubation had the most significant impact in reducing bacterial loads, *aadA*, and *sul1* per gram of litter. These processes had a significant effect on bacterial community composition. Changes in AMR gene abundance appears to reflect a shift in litter community structure.

# Ecology, Epidemiology & Environmental Microbiology

## Marcel Shams Eddin

ID IGEP cohort, Biological Sciences

Faculty Mentor: David Popham

Bacteria from the order 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. Yet, these spores are able to 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, contributing to rehydration of the spore and transition to the vegetative state. SleB has been shown to interact 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 docking to try to understand this type of interaction. To verify the interactions experimentally, site-specific mutagenesis will be used. YpeB is rapidly proteolyzed during spore germination. HtrC, a serine-type endopeptidase has been shown to degrade YpeB, but YpeB is still degraded in the absence of HtrC, showing that other proteases can be involved in the degradation process. Five putative proteases have been identified to be present in the spore inner membrane. Not only SleB and YpeB interact with each other, but they are also codependent on each other meaning that either of the two proteins are immediately degraded in the absence of their partner protein, an observation that ties my two projects together. We hypothesize that at least one of the five proteases will be serving HtrC's redundant role in YpeB degradation. How the degradation of YpeB affects germination is not fully understood. We hypothesize that YpeB degradation helps release SleB to degrade the peptidoglycan. However, if YpeB degradation does not affect germination, we hypothesize that the process of YpeB degradation is a clean-up process done by the spore to get rid of the protein after serving its purpose. 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 the germination, thus making the spores inefficient in causing human disease.

# Ecology, Epidemiology & Environmental Microbiology

## Forde Upshur

ID IGEP affiliated student

### Sugar-feeding by invasive mosquito species on ornamental plants

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Feeding on plant-derived sugars is an essential component of mosquito biology that affects key aspects of their lives such as survival, metabolism, and reproduction. Mosquitoes locate plants to feed on using olfactory and visual cues. *Aedes aegypti* and *Aedes albopictus* are two mosquito species invasive to the US, and are vectors of diseases such as dengue fever, chikungunya, and Zika. These species live in heavily-populated, urban areas, where they have a high accessibility to human hosts as well as to plants in backyards and town landscapes. Therefore, it is important to understand what plants may attract / repel mosquitoes to inform citizens and municipal authorities accordingly. Here, we observe *Ae. aegypti* and *Ae. albopictus* sugar-feeding behavior with eleven different commonly-planted ornamental plant species. We then assessed feeding activity using the anthrone method and identified volatile composition of plant headspace using gas-chromatography mass-spectroscopy. Finally, we determined the sugar-feeding activity of local mosquitoes using the plant DNA barcoding technique and compared these results with the eleven ornamental species tested in the lab.

# Human Dimension of Infectious Diseases, Public Health and Clinical Microbiology

## Amanda Darling

ID IGEP affiliated student, Civil & Environmental Engineering

Faculty Mentor: Alasdair Cohen

## Drinking water quality and associated health outcomes in rural Appalachia: A systematic review and meta-analysis

Amanda Darling<sup>1,2</sup>, Hannah Patton<sup>3</sup>, Md Rasheduzzaman<sup>1</sup>, Rachel Guevara<sup>4</sup>, Joshua McCray<sup>4</sup>, Leigh-Anne Krometis<sup>3</sup>, & Alasdair Cohen<sup>1,2\*</sup>

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**Background:** Of the approximately two million Americans who still lack reliable access to safe drinking water, many live in low-income rural areas of the Appalachian Region. Despite longstanding documentation of regional health disparities in rural Appalachia, aside from media coverage of high-profile instances of water contamination, available data on drinking water contamination and associated health outcomes in the region are limited.

**Objectives:** The objective of this study was to comprehensively review and synthesize published research findings on drinking water quality and associated health outcomes in rural Appalachia.

**Methods:** We conducted a systematic review of drinking water contaminants – microbiological and chemical – and drinking water related health outcomes in the Appalachian region. We pre-registered our study protocols limiting eligibility to studies that collected primary data in predominantly rural counties in the Appalachian region (as defined by the Appalachian Regional Commission). We searched four databases (PubMed, EMBASE, Web of Science, and the Cochrane Library) for records published over a 20-year period from January 1, 2000 to December 31, 2019. In addition to qualitative syntheses, meta-analyses (using sample size-based weighting), and risk of bias analysis, we conducted a meta-regression (generalized linear model with a logit link, binomial distribution, and publication/cluster-robust standardized errors) to assess the likelihood of chemical contaminants being reported as above US Environmental Protection Agency (EPA) Maximum Contaminant Limits (MCL).

**Results:** We identified 3,452 records for screening; 85 met our eligibility criteria and reported sufficient data for extraction. 56% of studies were conducted in Northern (32%, n=27) and North Central (24%, n=20) Appalachia, with only five studies (6%) identified from Central Appalachia. Across studies of different drinking water sources, *E. coli* were detected in 10.6% of samples (weighted mean percentage from 4,671 samples across 14 papers). Sample-size-weighted mean chemical contaminant concentrations were 0.010 mg/L for arsenic (from 6 papers, n = 21,262 samples), 0.001 mg/L for cadmium (2 papers, n = 21,061), 0.009 mg/L for lead (5 papers, n = 23,259), and 0.300 mg/L for uranium (4 papers, n = 36). The weighted mean concentration for PFOA was 103 ng/L (2 papers, n = 30). 93% of eligible publications (n = 79) were based on cross-sectional designs, and of the 27 publications reporting measured health outcomes only four were based on case-control or cohort study designs. The most commonly reported health outcomes were gastrointestinal illness (19%, n = 5 papers), detection of PFCs in blood serum (48%, n = 13), and cardiovascular-related outcomes (15%, n = 4).

**Conclusions:** Aside from extensive analyses of chemical contaminants in groundwater in northern areas of Appalachia, our findings reveal that there are relatively few published studies of drinking water sources, use, and quality in rural Appalachia. Our results demonstrate that more epidemiologic-based research is needed to better understand the nature and extent of exposures to contaminated drinking water and associated impacts on health in rural Appalachia.

# **Human Dimension of Infectious Diseases, Public Health and Clinical Microbiology**

## **Priyanka Theophilus**

Postdoc

Department: Biological Sciences

Faculty Mentor: David Popham

### **Characterizing the Inner Spore Membrane Lipid Composition in *Bacillus subtilis***

Bacterial endospores are the most resilient and persistent life forms on earth. Upon starvation, vegetative cells undergo systematic changes in gene expression that ultimately result in a metabolically inactive, multi-layer structure capable of withstanding a range of environmental insults including heat, desiccation, high pressure, radiation, and various toxic chemicals. Conversely, the dormant spores revert to vegetative growth in the presence of nutrients through the process of germination. The inner membrane (IM) of the endospore exhibits unique physical properties that are starkly different from its vegetative counterpart. Studies have shown that the spore IM is rigid and highly impermeable contributing to its resistance and dormancy. Interestingly, altering the lipid content of the spore IM was found to render the endospore susceptible to disruption or to stimulate early germination. Therefore, we speculate that the lipid composition of the spore IM plays an important role in maintaining membrane fluidity, directly contributing to the spore's resistance and germination properties. Proteome and TnSeq studies in our lab have revealed a list of genes suggested or known to be involved in lipid metabolism that are associated with the spore IM or with altered germination efficiency. Preliminary results show that genetically manipulating these genes in *Bacillus subtilis* did not affect growth or sporulation. Our goal is to identify strains that exhibit significant changes in the spore properties and to further characterize the lipid alterations in the spore IM of these mutant strains by HPLC-Mass spectrometry. Understanding the effects of spore IM lipid composition on spore stability and germination could establish novel methods to impart spore killing or accentuate germination thereby preventing human diseases.



# **Human Dimension of Infectious Diseases, Public Health and Clinical Microbiology**

## **Zachary Baker**

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Bryan Hsu

### **In situ production of therapeutically relevant proteins via T4 phage infection**

The prevalence of gastrointestinal disease (i.e. Ulcerative Colitis, Crohn's Disease, Obesity) has increased over the past few decades within the United States, and as a result, the demand for novel mechanisms of treatment is paramount. The delivery of therapeutics to the intestinal tract, however, has been notoriously difficult due to the harsh conditions present within the stomach, as well as noncompliance by patients. Recent research has proposed bacteria as a chassis for therapeutic protein delivery to the gut, yet is plagued by inefficiencies in colonization and treatment duration. Alternatively, we propose the use of phage to rehabilitate the gut microbiome, due to the coexistence of phage and bacteria in the GI tract, as well as the dispersal of cellular contents into the surrounding environment during cell lysis. We hypothesize that the lytic cycle can be harnessed to synthesize and disperse therapeutically relevant proteins, allowing for long-term treatment potential. Preliminary in vitro studies confirmed efficient distribution of protein to the surrounding environment through cell lysis, as well as significant production of phage-based GFP protein when integrated into the WT T4 phage genome. In vivo coexistence of engineered phage and bacteria within a murine model showed similar colonization to WT isolates, as well as visible GFP production within the mucosal lining. Subsequent integration of potential therapeutics, such as SERPIN B1a and ClpB, have also shown promise within this system for the amelioration of mammalian gastrointestinal diseases. This includes the in vitro inhibition of Neutrophil Elastase, linked to Ulcerative Colitis severity, as well as the in vivo reduction of food consumption and weight gain associated with Diet-induced Obesity. In summary, the production and release of therapeutic proteins via lytic phage, offers a promising avenue for treatment of gastrointestinal disease and a unique alternative for microbiome rehabilitation.

# Immunology & Host-Pathogen Interactions

## Rogério Bataglioli

Postdoc, Department: Biological Sciences

Faculty Mentor: Bryan Hsu

### Live microbial sentinels for the eradication of diarrheal-causing pathogens

Enteric bacterial pathogens are infectious agents commonly transmitted by the consumption of contaminated water and food. *Salmonella enterica* serovars Typhimurium and Enteritidis are the two most common nontyphoidal serovars that cause diarrheal diseases and lead to high death rates in developing countries. Whereas mild symptoms of salmonellosis do not require treatment, severely ill patients demand the administration of antibiotics. Despite the efficacy, many single or multidrug-resistant *Salmonella* clinical isolates have been reported worldwide, raising concerns about the spread of super-resistant strains. Bacteriophages (or phages) are the natural predators of bacteria and are a potential alternative antimicrobial strategy, one capable of precisely targeting bacterial pathogens in their native environment with minimal disruption to the gut microbiota. This study focuses on engineering commensal bacterial strains for mammalian gut colonization to prevent diarrhea by the exogenous triggered release of phages that predate enteric pathogens. The first step to accomplish this involves understanding whether P22 phage produced by commensal gut bacteria can infect *Salmonella enterica*, the targeted pathogen investigated herein. *Salmonella* encodes restriction-modification (RM) systems that recognize DNA methylation patterns and degrade unknown, heterologous genes for bacterial defense. Our recent findings indicate that the type-III RM system (StyLT1) in *Salmonella* substantially restricts the infection of P22 phage. The modified *E. coli* MG1655::pPR1347, carrying the genes for expressing the P22 lipopolysaccharide receptors, enables *E. coli* to harbor P22 prophage. However, the absence of the RM mechanism in *E. coli* genome leads to unmethylated phage particles that present a limited infection in *Salmonella*. To overcome this, *E. coli* MG1655::P22 has been engineered with StyLT1(Res-Mod+::CamR) in order to enhance the infectivity efficiency of P22 in the *Salmonella* wild type. Upcoming steps will involve integrating genome constructs that can activate P22 production, including the *cII* gene under the constitutive promoter ProC that represses the phage lytic cycle and the chemically triggered *cIIDN* gene (dominant negative) under the chemically activated pTet promoter. In the presence of anhydrotetracycline (aTc), the pTet promoter transcribes the *cIIDN* and induces the lytic cycle of P22 in *E. coli*. Overall, we aim to demonstrate that this engineered *E. coli* can work as sentinels in the mammalian gut to ameliorate diseases caused by enteric pathogens by targeting the causative microbial factors.

# Immunology & Host-Pathogen Interactions

## Rachel Bianculli

ID IGEP affiliated student, Chemistry

Faculty Mentor: Michael Schultz

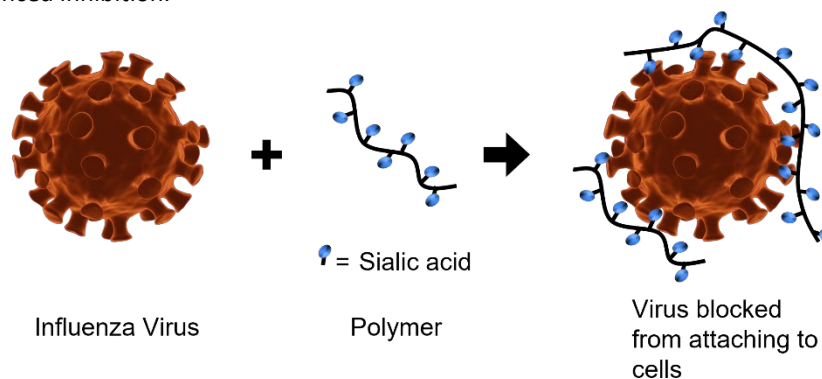
## IMITATION IS THE SINCEREST FORM OF FLATTERY: USING SIALIC ACID-CONTAINING POLYMERS FOR INFLUENZA INHIBITION

Rachel H. Bianculli, Jonathan D. Mase, Michael D. Schulz

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In nature, there are many examples of host-pathogen interactions that are polyvalent (viruses, bacteria, cells, etc.).<sup>1</sup> This concept of polyvalency, where multiple binding sites on the same surface of a host interacting with multiple targets on a pathogen have a synergistic effect and increase binding affinity, can be synthetically mimicked with polymers. Antiviral polymers, for example, target viral surfaces by acting as a decoy receptor for the virus to bind to. When antiviral polymers bind to the surface of the virus, the virus can no longer interact with the cell surface, and this lack of interaction reduces viral replication.

Influenza replication is initiated when hemagglutinin on the virus surface binds to sialic acid on the epithelial cell surface of the lungs. This same hemagglutinin-sialic acid interaction can be imitated synthetically by appending sialic acid to a polymer. We aim to make a library of sialic acid-containing polymers with systematically varied parameters (sialic acid content, molecular weight, and comonomer identity) and measure their influenza inhibition using a hemagglutination inhibition (HAI) assay. We will use this data to study structure-property relationships and optimize materials for enhanced inhibition.



**Figure 1. Sialic acid-containing polymers mimic nature and act as a “decoy” receptor for influenza to bind to. (Not drawn to scale)**

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# Immunology & Host-Pathogen Interactions

## Yuanzhi Bian

ID IGEP affiliated student, Biological Systems Engineering

Faculty Mentor: Mike Zhang

## Adjuvanticity of Interferon- $\gamma$ and Possible Synergy with Toll-Like Receptor Agonists

Yuanzhi Bian, Debra Walter, Chenming Zhang, Biological Systems Engineering

Interferon- $\gamma$  (IFN- $\gamma$ ) is a cytokine that plays an important role in immune regulation, especially in the activation and differentiation of immune cells. It has been studied in vaccines against various human and animal infectious diseases, including influenza and HIV-1, and demonstrated excellent adjuvant activity. Toll-like receptors (TLRs) are a family of pattern-recognition receptors that sense structural motifs related to pathogens and alert immune cells to the invasion. TLR agonists have been widely used as immunoadjuvants to augment the efficacy of cancer immunotherapies and vaccines against infectious diseases. To optimize the adjuvant formulation of a nanovaccine against oxycodone, we performed an *in-vitro* study to evaluate the adjuvanticity of IFN- $\gamma$  and TLR agonists. In brief, murine dendritic cells were treated with IFN- $\gamma$  and the TLR agonists, polyinosinic-polycytidylic acid (poly I:C; TLR3 agonist) or resiquimod (R848; TLR7/8 agonist). Subsequently, the cells were stained for CD86, an activation marker of dendritic cells, and CD86 expression was measured using flow cytometry. From the preliminary data, we found that IFN- $\gamma$  could efficiently activate the dendritic cells, while the TLR agonists by themselves could not. This was unexpected as the expression of TLR3, 7, and 8 by the dendritic cells was confirmed using reverse transcription-polymerase chain reaction. Interestingly, the combination of IFN- $\gamma$  with poly I:C or R848 triggered a higher amount of cell activation than IFN- $\gamma$  alone. 10 ng/mL IFN- $\gamma$  with 50  $\mu$ g/mL poly I:C or 100 ng/mL R848 achieved 47.9% and 52.8% cell activation, respectively. Both were significantly higher than the 36.8% positive cells obtained by 10 ng/mL IFN- $\gamma$  ( $p = 0.0145$  and  $0.0031$ , respectively). These results suggested that IFN- $\gamma$  and TLR agonists could be applied as a complementary, co-adjuvant system to promote dendritic cell activation and antigen presentation. Additionally, there might be a synergy between the two classes of adjuvants; further investigation is warranted to ascertain the interaction of their adjuvant activities.

# Immunology & Host-Pathogen Interactions

## Jules Dressler

Postdoc

### Elucidating the interactions between NapA-peptidoglycan and implications for the immune response

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*Borrelia burgdorferi*, the causative agent of Lyme disease, has characteristics that result in unusual interaction(s) with the human host compared to other bacterial pathogens. It lacks classical virulence factors and undergoes antigenic variation; two features that have hindered both diagnostic and vaccine development. In order to address these challenges, we have been investigating the peptidoglycan (PG) of *B. burgdorferi*. Since this bacterium lacks a PG recycling pathway, it consistently sheds a significant amount of PG into the host environment during infection. The PG of *B. burgdorferi* persists in the synovial fluid of Lyme arthritis patients, and these same patients produce long-lasting antibodies that are specific to the Lyme spirochete's cell wall. Recently, Neutrophil Attracting Protein A (NapA) has been discovered to be a PG-associated protein that plays an important role in protection against cell wall stress as well as modulation of the host immune response. We propose that the association between the two immunomodulatory molecules—NapA and PG—have implications for not only host immune response(s) during infection, but for Lyme disease diagnostic and vaccine development.

Here, we examined the immunomodulatory properties of NapA and its association with the *B. burgdorferi* sacculus. We then exploited this interaction to assess both the vaccine and diagnostic potential of covalently crosslinked NapA-PG antigens in live mice. Our findings provide new insights into the pathogenicity of NapA associated PG, and promising avenues to exploit this relationship as a means to diagnose and prevent Lyme disease.

# **Immunology & Host-Pathogen Interactions**

## **Hollyn Franklin**

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Bryan Hsu

### **Gut phages impact antibiotic response**

The impact of bacteriophages (phage) on the mammalian gut microbiome has been largely unexplored, with numerous studies focusing solely on the effects of the bacterial community. Recent advances in sequencing technology has allowed for the elucidation of the gut virome, as well as how it influences the microbial community and host health. Despite these advances, we are still limited in our ability to study the interactions between bacteria and phage in vivo. To better study these phenomena, we have developed a reliable method of depleting viral particles within the mammalian gut virome, through the inhibition of phage replication. The depletion of viral particles, and subsequent reconstitution of native bacteriophage, allow for the analysis of virome impact on murine health under unique gastrointestinal dysbiosis, including antibiotic perturbation. In summary, the presence or absence of phages in the gut microbiome is critical, impacting antibiotic response on human health and disease.

# Immunology & Host-Pathogen Interactions

## Brittany Heath

ID IGEP affiliated student, Biomedical and Veterinary Science

Faculty Mentor: Kyleene Kehn-Hall

## The role of glial inflammatory mediators on neurodegeneration induced by SARS-CoV-2 infection

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SARS-CoV-2, the etiological agent of COVID-19, has been shown to impact multiple organ systems including the nervous system, comprising the Central Nervous System and Peripheral Nervous System. Neurological manifestations have been reported in patients with symptoms ranging from anosmia (loss of smell) to encephalitis. The Central Nervous System (CNS) involvement in COVID-19 patients has been described to exhibit similar CNS alterations associated with neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease. There is a crucial need to understand the underlying mechanisms involved in CNS alteration and neurodegeneration due to SARS-CoV-2 infection. As an initial step to elucidating these pathways, we sought to characterize SARS-CoV-2 cellular tropism in glial cells (astrocytes/microglia) and neurons, and to identify secreted inflammatory mediators. Multiple cell types were infected with SARS-CoV-2 and evaluated up to 48 hours post-infection (hpi) for changes in release of infectious virus, viral RNA, and changes in gene expression. Infectious virus was detectable in human primary astrocytes up to 48 hpi, with a slight decline in viral titers from 24 to 48 hpi. In contrast, microglia displayed rapid clearance of infectious virus with viral titers falling below detectable limits 48 hpi. Intracellular viral RNA was detectable in both astrocytes and microglia up to 48 hpi, however viral RNA was approximately two logs higher in astrocytes compared to microglia at 24hpi. Our findings suggest astrocytes and microglia are susceptible to SARS-CoV-2 infection however do not result in the productive release of infectious viral particles. Ongoing studies aim to identify changes in gene expression associated with glial inflammatory mediators, including CXCL10, interleukin-1 $\beta$ , and interleukin 6.

# **Immunology & Host-Pathogen Interactions**

## **Hannah Ivester**

ID IGEP affiliated student, Translational Biology, Medicine and Health

Faculty Mentor: Coy Allen

## **Calming the storm: The role of the anti-inflammatory protein NLRX1 in the immune response to SARS-CoV-2**

SARS-CoV-2, the causative virus of the COVID-19 pandemic, has certainly impacted the world in major ways since its emergence in December 2019. One of the characteristic presentations of COVID-19 disease includes what has been termed the 'cytokine storm' of severe disease, as well as the Multisystem inflammatory Syndrome that is seen in pediatric patients. One of the functions of the immune system is to be able to modulate the response to mitigate host tissue damage as a result of an overzealous immune response. In cases of COVID-19 presenting with a 'cytokine storm,' this ability to balance the immune response appears to be lost to some degree, resulting in large amounts of tissue damage and more severe disease outcomes. One of the proteins responsible for negative regulation of the immune response is the protein NLRX1. Here we show that deficiencies in NLRX1 result in differential immune responses to SARS-CoV-2 both in cell culture and in mice, showing differences in viral clearance, disease presentation, and cytokine production. In addition, further data might suggest that this protein could offer itself as a therapeutic target to help mitigate the effects of the 'cytokine storm' related to severe COVID-19 disease. Finally, this data also adds to the growing body of evidence related to SARS-CoV-2 and its pathobiology in an understudied context of immune regulation and its importance in antiviral immunity.



# Immunology & Host-Pathogen Interactions

## Jonathan Joyce

ID IGEP affiliated student, Translational Biology, Medicine & Health

Faculty Mentor: Andrea Bertke

## **SARS-CoV-2 Rapidly Infects the Peripheral and Central Nervous Systems of Mice and Hamsters via Neuronal Entry Facilitated by Neuropilin-1 Before Detectable Viremia**

Jonathan D. Joyce<sup>1,6</sup>, Greyson A. Moore<sup>2</sup>, Poorna Goswami<sup>1</sup>, Telvin Harrell<sup>2</sup>, Emma H. Leslie<sup>1</sup>, Seth A. Hawks<sup>3</sup>, Christopher K. Thompson<sup>4</sup>, Nisha K. Duggal<sup>3</sup>, and Andrea S. Bertke<sup>5,6</sup>

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Up to 80% of patients with acute COVID-19 experience neurological symptoms. These symptoms can last long into recovery with 85% of those with post-acute sequelae of SARS-CoV-2 reporting them. Detection of viral RNA, protein, and virus in the cerebrospinal fluid and brains of COVID-19 patients indicates that SARS-CoV-2, the causative virus, is neuroinvasive. Although studies have focused on the ability of SARS-CoV-2 to invade the central nervous system (CNS) through infection of neurons responsible for smell, little attention has been paid to the ability of SARS-CoV-2 to infect the peripheral nervous system (PNS) or other neural pathways connecting with the CNS. Additionally, the role of direct neural entry vs hematogenous entry, viral replication dynamics in neurons, and potential viral receptors beyond ACE2 have yet to be assessed. To assess the ability of SARS-CoV-2 to infect the PNS as well as to determine the role of direct neuronal entry vs hematogenous entry we intranasally inoculated K18-hACE2 mice (n=35), wild-type mice (n=34), and hamsters (n=10) with SARS-CoV-2 isolate USA-WA1/2020. Peripheral nervous system tissues (sensory: dorsal root (DRG), trigeminal (TG) ganglia; autonomic: superior cervical ganglia (SCG)), brain regions (olfactory bulb, cortex, hippocampus, brainstem, cerebellum), whole brain hemispheres, and spinal cord samples were collected 18-, 42-hrs, three-, and six-days post infection. Viral RNA was detected using RT-qPCR, antigen and dsRNA via immunostaining, and infectious virus via plaque assay. Viral RNA, protein, and infectious virus was found in the PNS, spinal cord, brain regions, and neuronal supporting cells as early as 18 hours post infection, preceding viremia. Primary neuronal cultures from K18-hACE2 and wild-type mice were infected and assessed as above to determine SARS-CoV-2 replication kinetics in neurons. Viral RNA increased over time in a neuron dependent manner and dsRNA was observed indicating viral replication. When neuronal cultures were pretreated with the neuropilin-1 (NRP-1) antagonist EG00229 prior to infection, viral RNA in DRGs was reduced by 99.8% and 86.7% in hACE2 and WT DRGs, respectively. We show that sensory and autonomic ganglia of the peripheral nervous system, neuron supporting cells, discrete brain regions, and the spinal cord are permissive to infection, that neuroinvasion occurs rapidly via direct neuronal entry preceding viremia, and that neuronal entry involves NRP-1. Our results indicate that SARS-CoV-2 can invade and establish a productive infection in previously unassessed sites in the nervous system. Invasion of these tissues may contribute to neurological symptoms of COVID-19 and merit further investigation as COVID-19 transitions from a pandemic disease to an endemic disease.

# Immunology & Host-Pathogen Interactions

## Rita Makhoul

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Bryan Hsu

### Investigating the Role of Virulent Phages in Ameliorating Murine Colitis

Inflammatory Bowel Disease (IBD) refers to a category of chronic diseases characterized by the inflammation of the gastrointestinal (GI) tract. IBD comprises two main diseases: Crohn's disease (CD), referring to the inflammation of the bowel wall in any part of the GI tract; and Ulcerative Colitis (UC) defined by the specific inflammation of the innermost lining of the colon and/or rectum. Currently, IBD affects approximately 1.6 million Americans with 70,000 new cases diagnosed each year. Although the exact etiology of this disease is not fully elucidated, a direct link has been established between the composition of the intestinal microbiome and the pathogenesis of IBD. Most of the studies aiming at elucidating the role of the gut microbiota in the onset of IBD focus on the bacterial community. Nevertheless, increasing evidence suggests that the gut phage consortium plays a crucial role in shaping and regulating the gut microenvironment. Additionally, dysbiosis in the gut virome, predominantly bacteriophages, has been observed in IBD patients.

The probiotic *Escherichia coli* strain Nissle 1917 has been used in the treatment of chronic inflammatory diseases, however its exact mechanism of action remains obscure. Using the chemical (Dextran sulfate sodium, DSS) murine colitis model, we have discovered that the co-colonization with a lytic phage for this bacterium ameliorates colitis, but the absence of this phage, or the presence of another strain of lytic phage does not. Alternative to previous findings that phages can exacerbate intestinal colitis, we find that phages can have an ameliorative effect. This has broad implications for the functional importance of the gut virome in not just dysbiosis but also symbiosis.

# Immunology & Host-Pathogen Interactions

## Jon Mase

ID IGEP affiliated student, Chemistry

Faculty Mentor: Michael Schultz

Norovirus is a global health burden that infects nearly 700 million people annually. Research into vaccines and antivirals has met numerous hurdles and to date no medication exists on the market to combat the virus. Antiviral polymers may be able to fill that gap. To bind viruses onto a polymer's surface, common glycans norovirus utilizes for human infections will be synthesized. In this work, modified sialic acids (SAs) are chemically synthesized and equipped with an acrylamide linker on C2. Histo-blood group antigens (HBGAs), possessing a more complex glycan structure, are designed by combining dual synthetic routes: chemical synthesis and automated chemical synthesis via automated glycan assembly. Hyperbranched materials are synthesized using reversible addition-fragmentation chain-transfer (RAFT) polymerization on a tetrafluorophenyl 4-vinylbenzene sulfonate monomer. The effect of polymer architecture will be investigated by varying the molecular weight and degree of branching to create a library of materials. The presence of terminal trithiocarbonate functional groups on the hyperbranched structures allows for the controlled and selective insertion of glycans (SA or HBGAs) onto the terminal ends of polymers. Antiviral inhibition will be measured using emagglutination assays.

# Immunology & Host-Pathogen Interactions

## Mecaila McClune

ID IGEP affiliated student, Biochemistry

Faculty Mentor: Brandon Jutras

## Investigating the relationship between *Borrelia burgdorferi* peptidoglycan and Lyme arthritis

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Lyme disease is the most common vector-borne illness in the United States with an estimated 500,000 new cases each year. If left untreated this disease can cause debilitating symptoms such as arthritis, carditis, and neuroborreliosis. Lyme arthritis is the most common late-stage symptom of Lyme disease affecting around 50,000 people each year. Recent studies suggest a link between Lyme arthritis and a particular cell wall component of the causative bacterial agent, *Borrelia burgdorferi*. This component, peptidoglycan (PG), is composed of peptides and glycan strands and provides structural support to the cell. It has also been shown to have immunostimulatory effects, acting as a Pathogen Associated Molecular Pattern (PAMP). Not only does *B. burgdorferi* lack the enzymes and transporters necessary to recycle PG as they grow and divide, but *B. burgdorferi* PG has a unique chemical structure in comparison to other types of bacteria. This results in the release of ~45% of *B. burgdorferi*'s total PG into its environment each generation. *B. burgdorferi* PG has even been detected in the synovial fluid of Lyme arthritis patients post antibiotic treatment. If purified *B. burgdorferi* PG is also able to mimic Lyme arthritis when injected into a murine model. We hypothesize that *B. burgdorferi* PG has an increased half-life *in vivo*, unusual biodistribution, and induces unique transcriptomic responses. To test this, PG was purified from various bacteria that all differ in PG structure, *B. burgdorferi* included. The PG was then fluorescently labeled, injected into mice, and a real-time *in vivo* imaging system was used to track its biodistribution over time. Through these studies we found that *B. burgdorferi* PG persists for extended periods of time *in vivo*, specifically within the liver, using IHC to confirm this localization. Through bulk RNA-sequencing we have also shown that *B. burgdorferi* PG induces unique gene expression pathways in human peripheral blood mononuclear cells (PBMCs) in comparison to other bacterial PG. These results implicate the involvement of PG in Lyme arthritis and serves as a suitable target for better treatment and therapeutic approaches.

# Immunology & Host-Pathogen Interactions

## Charlotte Nyblade

ID IGEP affiliated student, Biomedical Sciences and Pathobiology

Faculty Mentor: Lijuan Yuan

### **A new gnotobiotic pig model of P[6] human rotavirus infection and disease**

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Human rotavirus (HRV) is a leading cause of gastroenteritis in children under 5 years of age. Licensed vaccines containing G1P[8] and G1-4P[8] strains have been available since 2006, however they are less efficacious against newly emerging P[6] strains. This indicates a clear need to develop vaccines that can protect against P[6] genotypes. In the past, vaccine development has been aided by the gnotobiotic (Gn) pig model of G1P[8] Wa HRV infection and disease. Here, we aimed to develop a new Gn pig model of P[6] HRV infection and disease to support the evaluation of potential vaccine candidates. The Arg HRV (G4P[6]) strain was derived from an infant stool sample. For virus adaptation and amplification, neonatal Gn pigs were orally inoculated with stool containing  $5.6 \times 10^6$  fluorescent focus units (FFU)/mL of the virus. Small intestinal contents were collected at post inoculation day (PID) 2 or 3. The virus was passaged 6 times in neonatal Gn pigs, resulting in an inoculum pool with a titer of  $3.4 \times 10^5$  FFU/mL. Next, 33-34 day old Gn pigs were orally inoculated with 0.01,  $10^3$ ,  $10^4$ , and  $10^5$  FFU of Arg HRV to determine the optimal challenge dose. Rectal swabs were collected daily to detect virus shedding and diarrhea. All pigs were infected, with the onset of virus shedding and diarrhea occurring between PID1-4. In the lowest dose group, the mean duration of virus shedding and diarrhea was 3 days. In the higher dose groups, pigs shed virus and had diarrhea for 4-6 days. By PID7, all pigs had resolved infection and disease. The optimal challenge dose was determined to be  $10^5$  FFU which was comparable to the optimal challenge dose of virulent Wa HRV in Gn pigs. The severity and duration of diarrhea and virus shedding in Arg HRV infected pigs were also similar to that seen in Wa HRV infection. This model is ready to support the process of evaluating candidate multivalent HRV vaccines.

# Immunology & Host-Pathogen Interactions

**Asifur Rahman**

ID IGEP affiliated student,

## **Investigating Bacteriophage-Host Interaction Using Raman Spectroscopy Combined with Stable Isotope Labeling**

Asifur Rahman, Wei Wang, Peter J. Vikesland

The metabolic activity of bacteria is fundamental to many environmental processes, such as biogeochemical cycling, disease marker propagation, biofilm development, etc. Bacteriophages exclusively infect bacteria and affect their metabolism, which has important implications for phage-based biocontrol in several fields, such as public health, food safety, therapeutics, sustainable energy productions, etc. Raman spectroscopy has become an essential tool for biomolecular analysis due to its sensitive detection and non-destructive application. Stable isotope labeling involves supplementing microorganisms with nonradioactive isotopes (e.g.,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ) for incorporation of these isotopes into metabolically active microorganisms. The combination of Raman spectroscopy with stable isotope labeling has become a widely used technique to investigate biochemical processes. This study is the first to describe the application of Raman- stable isotope technique to investigate the dynamics of Bacteriophage-Host Interaction. In this study, we investigated how viral infection affects the uptake of deuterium (D) isotope by the bacterial strains *Escherichia coli* C3000 and *Pseudomonas syringae*. Both strains were observed to have a C-D stretching vibration peak ( $2100\text{-}2300\text{ cm}^{-1}$ ) in the Raman spectrum when cultured in a D labeled growth media. Bacterial growth as well as the C-D peak intensity were monitored following exposure to organism specific bacteriophages (MS2 and Phi6, respectively). The average of the absolute Raman intensities of the C-D band of the viral infected bacteria samples was  $\sim 3\times$  lower for MS2 infection and almost  $\sim 22\times$  lower for Phi6 infection, when compared to the control with no virus infection. These results suggested that viral infection leads to a reduction of bacterial growth, hence resulting in inhibited metabolic activity and D uptake. The time- dependent Raman spectra of the MS2 and Phi6 infected bacteria *E. coli* C3000 and *P. syringae* showed the intensity of the C-D signal over time, which was consistent with their OD600 growth kinetics. A mostly linear correlation was observed between the absolute intensities of the C-D peak in the Raman spectrum and the initial  $\log_{10}$  concentrations (PFU/mL) of the bacteriophages Phi6 and MS2. Overall, the Raman-stable isotope technique can be a promising approach to examine the effects of environmental factors, such as viral infection on bacterial growth.

# Immunology & Host-Pathogen Interactions

## Safoura Salar

ID IGEP affiliated student, Biological Sciences

Faculty Mentor: Florian Schubot

## 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.

# Immunology & Host-Pathogen Interactions

## Parkesh Suseendran

ID IGEP affiliated student, School of Plant and Environmental Sciences

Faculty Mentor: Wang

## Identification of host genes restricting the replication of brome mosaic virus in dicotyledon plants cells

Parkesh Suseendran, Wenhao Zhao, and Xiaofeng Wang

Brome mosaic virus (BMV) is a positive-strand RNA virus that infects multiple monocot crops, such as barley, rice, and wheat. BMV has been used as a model virus due to its small genome and robust replication, as well as its ability to replicate in the baker's yeast. Although much knowledge in gene expression, genome replication mechanisms, and virus-host interactions has been gained by using the BMV-yeast system, there remains much to be learned about plant genes that restrict BMV replication in dicotyledon plant cells. Wild-type *Arabidopsis thaliana*, which is a model plant, is not a systemic host for BMV, but all *Arabidopsis* mutants with a dysfunctional *CPR5* gene allow for BMV infection. We set to identify more *Arabidopsis* mutants that allow for BMV replication by using Western blotting and tissue printing to detect BMV coat protein. Several host genes, which are coexpressed with *CPR5* or whose expressions are affected in *CPR5* dysfunction mutants, have been identified as critical to block BMV infection in *Arabidopsis*. This aids in our understanding of how dicot plants defend themselves from BMV infection and identification of restriction factors for potential virus control in monocot crops.



# Immunology & Host-Pathogen Interactions

## Juselyn Tupik

ID IGEP affiliated student, Biomedical Sciences & Pathobiology

Faculty Mentor: Coy Allen

### **“Striking a Balance”: Investigating homeostasis in NOD-Like Receptor (NLR) innate immune signaling during brucellosis**

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Brucellosis (*Brucella* spp.) is a bacterial zoonotic disease transmitted by livestock and is characterized by immune evasion. By hiding inside of macrophage cell vacuoles, *Brucella* often persist in human and animal hosts and, consequently, instigate chronic inflammatory conditions. Despite mechanisms to combat host immune defense, the innate immune system through pattern recognition receptors (PRRs) play an important role in immunoregulation during brucellosis. We have previously shown that formation of the pro-inflammatory NOD-like Receptor (NLR) class of PRRs into a multi-protein complex termed the inflammasome plays a protective role against brucellosis. This results from inflammasome activation of inflammatory cell death following recognition of *Brucella* genomic (g)DNA. However, the role of anti-inflammatory NLRs such as NLRX1 during brucellosis has not been determined. Here, we infected wildtype (WT) and *Nlrp1*<sup>-/-</sup> mice with *Brucella abortus* to evaluate the effect of an anti-inflammatory NLR during brucellosis. We found that WT mice displayed a stronger phenotypic presentation of infection, exhibiting increased splenomegaly and disease severity in the spleen, as well as elevated bacterial load in the liver when compared with knockout mice. These results indicate that NLRX1 may aggravate brucellosis presentation *in vivo*. Through further *in vitro* studies infecting murine WT and *Nlrp1*<sup>-/-</sup> macrophages, we found that NLRX1 attenuated inflammation, specifically in response to *Brucella* gDNA, but led to increased bacterial load in WT macrophages. To further determine potential mechanisms of NLRX1 activation, we also conducted these studies using novel NLRX1 overexpression (*NLRX1*<sup>OE</sup>) and control (*NLRX1*<sup>OE-CTL</sup>) THP-1 human monocytes and macrophages. These THP-1 cells contain two inducible reporters for Type I IFN and NF-κB inflammatory signaling, both pathways suggested to be regulated by NLRX1 downstream of bacterial infection. In these studies, we found that NLRX1 may modulate NF-κB signaling and cell death pathways in order to further exacerbate brucellosis infection. Ultimately, these studies emphasize the need to characterize NLRX1's mechanism of activation during brucellosis. Further, our investigation suggests the multifaceted role of NLRs in establishing immune system homeostasis in NLR-mediated diseases.

# Immunology & Host-Pathogen Interactions

## Morgen VanderGiessen

ID IGEP cohort, Biomedical & Veterinary Sciences

Faculty Mentor: Kylene Kehn-Hall

## Venezuelan equine encephalitis virus replication is impacted by p53 modulation

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Venezuelan equine encephalitis virus (VEEV) is a new world alphavirus that can cause mild flu-like illness which can progress into encephalitis in both humans and equines. VEEV capsid has a variety of functions including binding to membrane glycoproteins, inhibiting transcription of host cells, and blocking nucleocytoplasmic transport. Interestingly, the capsid protein of some other encephalitic viruses including West Nile virus has been demonstrated to mediate apoptosis through p53 activation, leading to higher pathogenicity. Several viruses including *Orthomyxoviridae*, *Flaviviridae*, and *Retroviridae* have been demonstrated to reduce p53 cell apoptosis pathway activity and/or develop alternative pathways to avoid cell apoptosis under viral stress. Here we investigate the relationship between VEEV capsid protein and p53 to identify whether VEEV capsid protein utilizes the p53 pathway to enhance viral replication. Initial studies consisting of LC-MS/MS analysis of capsid immunoprecipitated samples from VEEV infected cells identified p53 as a potential host interactor of VEEV capsid. To investigate p53 expression in neuronal cell types, we performed western blotting to confirm p53 expression in microglia cells (HMC3s) and astrocytes (U87MGs) and co-immunoprecipitation with a VEEV TC-83 V5 tagged capsid virus to verify the interaction between p53 and capsid. Furthermore, we used TaqMan gene expression assays to determine an upregulation of several p53 associated genes in cells infected with a VEEV TC-83 capsid mutant virus. To explore the impact of p53 on VEEV production, two small molecule modulators of p53, NVP-CGM097 and Pifithrin- $\alpha$  were utilized. NVP-CGM097 activates p53 via disruption of MDM2's interaction with p53. Pifithrin- $\alpha$  is an inhibitor of p53 dependent gene expression. Treatment of VEEV infected cells with both the p53 activator, NVP-CGM097, and the p53 inhibitor, Pifithrin- $\alpha$  at nontoxic concentrations (>80% cell viability) resulted in 4-5 log<sub>10</sub> reduction in viral titers. These studies indicate that modulation of p53 activity impacts VEEV replication. Ongoing studies are aimed at determining the impact of VEEV capsid on p53 responsive genes and depleting p53 using siRNA to confirm the importance of p53 for VEEV replication.

# Immunology & Host-Pathogen Interactions

## Wenhao Zhao

Postdoc, Department: School of Plant and Environmental Sciences

Faculty Mentor: Xiaofeng Wang

### **Host CPR5 genes play dual roles in nonhost resistance and susceptibility to a plant virus**

Brome mosaic virus (BMV) infects monocotyledon plants, such as barley, wheat, and rice, but not dicotyledon plants. Host determinants underlying nonhost resistance in dicots and susceptibility in monocots to BMV are unclear. It was reported that BMV systemically infects multiple Arabidopsis mutants with the *CPR5* gene disrupted, suggesting a role of *AtCPR5* in determining Arabidopsis as a non-host. However, mechanisms by which *AtCPR5* blocks but monocot *CPR5* promotes BMV infection remain unclear.

While Arabidopsis and many dicots have a single *CPR5*, dicots have two *CPR5* homologs in their genome. We cloned two *CPR5* genes from barley, *HvCPR5-1* & *-2*. Similar to *AtCPR5*, both *HvCPR5* proteins contain five transmembrane domains (TMDs) at the C-terminus. Using the antiserum recognizing each *HvCPR5-1* or *-2*, we found that accumulated *HvCPR5-1* decreased but *HvCPR5-2* increased during BMV infection in barley plants. We further found that *HvCPR5-2*, but not *HvCPR5-1*, was co-immunoprecipitated with BMV replication proteins 1a and replicase 2a<sup>pol</sup>. Our data suggest that barley *CPR5-2* may play a critical role in BMV replication.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Azahar Ali

Assistant Professor, Animal and Poultry Sciences  
College of Agriculture & Life Sciences

## Miniaturized Biosensors for Rapid Identification of Swine Hepatitis E Virus

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Hepatitis E is an inflammation of the liver caused by the hepatitis E virus (HEV).<sup>1</sup> Swine is believed to be the main reservoir of genotypes 3 and 4 (among four genotypes) of the human pathogen HEV.<sup>2</sup> It is noted that HEV is dominant at a high level in swine herds globally, indicating a high chance of transmission to humans.<sup>2</sup> The U.S. swine HEV isolate is known to be genetically related to the U.S. human HEV strains. Swine-to-human transmission of this virus occurred *via* the fecal-oral route. According to WHO, each year there are 20 million infections globally, leading to an estimated 3.3 million cases of HEV. Further, it is predicted that HEV caused 44000 deaths (~3.3% of the mortality rate) per year. It is thus important for its early detection to isolate and prevent the disease before its transmission to humans. At the CeZAP Infectious Diseases Symposium, I will discuss my future work on biosensors for the rapid detection of zoonotic diseases. The ultimate goal of this work is to pave the way toward a smartphone-enabled 3D- printed nano-architected lab-on-a-chip biosensor that can detect zoonotic disease *i.e.* HEV in pig samples.<sup>3</sup> This research will investigate a completely new biosensing concept: the integration of a 3D-printed electrode with multi-length-scale architecture, a microfluidic device, and a wearable platform. The main motivation of the work is to address challenges with the existing technologies for virus sensing by conducting research that will lead to (a) a wearable platform for on-site testing, (b) a low-cost manufacturing method, and (c) a highly sensitive and selective 3D array electrode. With this biosensor, it is expected that producers or farmers can improve herd health management and rely on animal-side testing to draw time-sensitive decisions. Further, I will also pursue to develop a set of biosensor devices for the detection of other zoonotic diseases in cattle such as Q fever, *Escherichia coli*, and Salmonellosis.

Keywords: Biosensors, 3D array electrode, Microfluidic devices, Hepatitis E Virus

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# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Aaron Brock

ID IGEP affiliated student, Biochemistry

Faculty Mentor: Brandon Jutras

## The Role of Motility in the Thickness of *Borrelia burgdorferi* Peptidoglycan

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Lyme disease is the leading tick-borne disease worldwide; however, little is known about its pathogenesis. The causative agent, *Borrelia burgdorferi*, does not secrete toxins or other typical virulence factors to cause infection. Therefore, this pathogen relies on other factors to facilitate infection, like motility. *B. burgdorferi* uses periplasmic flagella that wrap around the cell cylinder to move through their environment at high speeds. This mode of motility creates immense torsional stress on the poles of the cell cylinder. While many aspects of flagellar assembly and function are well described, the mechanism(s) that counteract flagellar torque, thus contributing to motility, are largely unknown but are thought to involve the peptidoglycan. Peptidoglycan is a large polymer complex within the periplasm, composed of long sugar chains cross-linked by short peptides containing L- and D-amino acids. Earlier studies found that *B. burgdorferi* uniquely synthesizes peptidoglycan through several zones of elongation. A mother cell is born with a single mid-cell zone of peptidoglycan synthesis, and as the cell cycle progresses, equidistant secondary and tertiary zones are created. The primary zone becomes the site of cell division, while the others dictate septation in the following generation. However, the mechanism(s) that underlies this process is entirely unknown. Since the zones of peptidoglycan synthesis become the poles of new-borne cells and are where the flagella become anchored, we hypothesized that motility plays a vital role in regulating peptidoglycan synthesis. Our studies show that the peptidoglycan at the poles is significantly thicker than the rest of the sacculi, indicating that zones of growth are both sites that prime cell division while reinforcing the cell wall. Analysis of several mutants deficient in flagellum assembly indicates that the motors play a crucial role in regulating *B. burgdorferi* peptidoglycan biosynthesis. Our findings could be exploited to create novel and specific therapeutics to target peptidoglycan synthesis, motility, or both — two essential components in *Borreliae* pathogenesis. More broadly, our studies reveal the first example of direct alterations in peptidoglycan thickness within a single cell and further our understanding of the unusual Spirochete phylum.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Chelsea Cereghino

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Faculty Mentor: James Weger-Lucarelli

Adaptation to mosquito vectors suited for transmission in urban settings is a major driver in the emergence of arboviruses. To better anticipate future emergence events, it is crucial to assess their potential to adapt to new vector hosts. In this work, we used two different experimental evolution approaches to study the adaptation process of an emerging alphavirus, Mayaro virus (MAYV), to *Aedes aegypti*, an urban mosquito vector of many arboviruses. We identified E2-T179N as a key mutation increasing MAYV replication in insect cells and enhancing transmission by live *Aedes aegypti*. In contrast, this mutation decreased viral replication and binding in human fibroblasts, a primary cellular target of MAYV in humans. When this mutation was introduced in the closely related chikungunya virus, which has caused major outbreaks globally in the past two decades, we observed increased replication in both human and insect cells, suggesting E2 position 179 is an important determinant of alphavirus host-adaptation, although in a virus-specific manner. We also showed that MAYV E2-T179N was attenuated *in vivo* in a mouse model. We then used structural and experimental analyses to show that MAYV E2-T179N bound less efficiently to human cells, though the decrease in replication or binding was not mediated through interaction with the mammalian receptor Mxra8. As such, we explored other mammalian receptors and entry factors which may be responsible for the attenuation of MAYV E2-T179N through competition binding assays. Collectively, these results indicate that adaptation at the T179 residue in MAYV E2 may result in increased vector competence of *A. aegypti* – but coming at the cost of optimal replication in humans – and may represent a first step towards a future emergence event.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

**Maria Dorodnitsyna**

ID IGEP affiliated student

## **PDGF/VEGF-related receptor PVR in mosquitoes is involved in juvenile hormone signaling from the plasma membrane**

Infectious diseases like malaria and zika fever are transmitted from female mosquitoes to humans through blood feeding as part of their reproductive cycle and are a leading cause of death in many developing countries. Growing mosquito resistance to pesticides currently on the market creates an urgency to investigate mosquito biology to find new targets that can be used for disease control. Juvenile hormone (JH) plays an essential role in mosquito egg maturation, acting via an intracellular receptor and a membrane receptor. The membrane receptor that binds JH to initiate the signaling pathway has not yet been identified. Our preliminary study suggests that PDGF/VEGF related receptor PVR is a top candidate for this role. To test this hypothesis, we performed RNAi-mediated knockdown of PVR in adult mosquitoes and CRISPR knockout in mosquito cell culture and measured JH-induced gene expression and protein phosphorylation. qPCR analysis indicates that PVR depletion caused a significant reduction in expression of the JH-controlled Krüppel-homolog 1 gene both in vivo and in vitro. Using western blot we observed that the JH-regulated phosphorylation of Serine/arginine-rich splicing factors was weakened after PVR knockout. Knockdown of PVR also repressed oocyte growth in adult females. These results suggest that PVR is indeed involved in juvenile hormone signaling. The elucidation of the juvenile hormone signaling pathway, especially the receptor binding JH, can facilitate the development of new pesticides with high mosquito specificity for disease control in the future.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Katara Griffith

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*Aedes aegypti* mosquitoes are a major vector for transmitting infectious diseases such as yellow fever, dengue, chikungunya, and Zika. Male mosquitoes do not bite, while female mosquitoes are responsible for disease transmission. A better understanding of mosquito reproduction will reveal effective targets for transmission control. Kruppel homolog 1 (Kr-h1), a gene that is regulated by both 20-hydroxyecdysone (20E) and juvenile hormone, is active throughout post-embryonic development. Kr-h1 is also required for proper expression of 20E-responsive genes in female mosquitoes during egg production, following blood feeding. This transcription factor has previously been identified as either an activator or repressor for the expression of various hormone-responsive genes, though its molecular mechanism remains largely unknown. Here we use the Cleavage Under Target and Release Using Nuclease (CUT&RUN) technique to examine genome-wide Kr-h1 binding profiles throughout the egg maturation process in adult female mosquitoes. The results not only verified Kr-h1 binding sites previously identified by chromatin immunoprecipitation assays but also open the door to defining the role of Kr-h1 in modulating chromatin accessibility of its target genes in female mosquitoes.



# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Jeffrey Marano

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Faculty Mentor: James Weger-Lucarelli

### **Impacts of cross-reactive flavivirus serum on ZIKV evolution, neutralization sensitivity, and fitness.**

Flaviviruses, such as dengue (DENV) and Zika virus (ZIKV), cause recurrent febrile and hemorrhagic disease resulting in 400 million infections annually. These viruses are in a constant arms race with their host's immune system, which drives continuous evolution. Due to co-circulation, these viruses must respond to the specific immune pressures and pre-existing immunity to closely related co-circulating pathogens. However, the impact of pre-existing cross-reactive immunity as a driver of evolution is not well understood, leaving us underprepared to handle the emergence of cross-reactive immune escape variants.

Therefore, this study investigated cross-reactive anti-DENV antibodies' role as drivers of evolution in ZIKV infection. We used an in vitro evolution system to passage ZIKV in mammalian cells with antibodies from patients previously infected with DENV (anti-DENV serum) or antibodies from DENV-naïve patients (control serum). Following five passages, next-generation sequencing was performed to identify any mutations within the viral populations. Twenty-one mutations were detected with an allelic frequency greater than 5%. A subset of the non-synonymous mutations found in common antibody targets (M, E, and NS1) was inserted individually into a ZIKV infectious clone to assess their effects.

The mutation found in NS1 (NS1-T139A) showed an escape phenotype, while the mutation in E (E-V355I) had sensitized to anti-DENV serum. Both viruses showed enhanced fitness when assessed in Vero cells with and without supplementation of anti-DENV serum. This fitness increase held in both human lung epithelial cells (A549) and human monocyte (U937-DC-SIGN) cells. When assessed in *Aedes aegypti* mosquitos, regardless of the immune status of the bloodmeal, the mutant viruses showed significantly reduced fitness compared to wild-type. These results indicate that while mutants can arise from cross-reactive immune selection, these mutations are unlikely to be sustained in human-mosquito-human transmission, which aligns with previous findings concerning the two host-hypothesis. Therefore, there still exists a need to understand what is driving the evolution of ZIKV in nature.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Samuel Rodgers

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Faculty Mentor: James Weger-Lucarelli

### Development of a Human Porcine Deltacoronavirus Infectious Clone

Coronaviruses have caused several devastating epidemics over the past 20 years, including the ongoing SARS-CoV-2 pandemic. Zoonotic transmissions from animals to humans has initiated each of the previous coronavirus outbreaks, demonstrating the need to study these viruses before spillovers occur. Previously, of the four genera of coronavirus, only *Alphacoronaviruses* and *Betacoronaviruses* had been shown to infect humans. However, in 2020, researchers found evidence of Porcine Deltacoronavirus spillover into the human population and infection of at least 3 children in different regions of Haiti. Two different strains of Human Porcine Deltacoronavirus (HuPDCoV) were identified in the Haitian children, which could either be explained by two separate infections, or human to human transmission. The aim of this project is to create an infectious clone of HuPDCoV to understand the viral genetic determinants that enabled HuPDCoV to efficiently infect humans; in the long term, this infectious clone will enable studies to inform on the possibility of a mass spillover and identify whether there is evidence of HuPDCoV adaptation to humans. Coronaviruses have historically been difficult to clone due to bacterial toxicity in the hosts, which are required to propagate plasmids in conventional cloning systems. Using the novel *in vitro* cloning system, Replication Cycle Reaction (RCR), we have eliminated the need for a bacterial host, making the process of cloning Coronaviruses significantly more efficient. This is because the lack of a bacterial host enables us to create novel infectious clones without unwanted, host-derived mutations. We can next compare growth *in vitro* to ensure the behavior of the recombinant virus matches the parental isolate. Aside from providing information on the HuPDCoV virus and the mechanisms of its spillover, this novel infectious clone provides a potential platform for a vaccine in the event of a future epidemic.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Manette Tanelus

ID IGEP affiliated student, Entomology

Faculty Mentor: Jonathan Auguste

### Evaluating the safety and efficacy of a novel chimeric Zika vaccine

Manette Tanelus, Krisangel López, Shaan Smith, John A. Muller, Danielle L. Porier, Dawn I. Auguste, William B. Stone, and Albert J. Auguste

Within the *Flaviviridae* family, there are mosquito-borne, tick-borne, arthropod-specific, and some viruses have no-known vectors clades. Zika (ZIKV) is a positive sense mosquito-borne flavivirus discovered with a history of sporadic outbreaks throughout Africa and the Americas. Currently Zika has been reported in eighty-six countries worldwide and has been associated with both Guillain Barré syndrome in adults and congenital Zika syndrome in infants. Given the apathogenic nature to vertebrates, insect-specific flaviviruses (ISFVs) are relatively understudied. Aripo virus is an ISFV that genetically clusters with pathogenic flaviviruses; like Zika. We developed an Aripo-Zika (AZ) chimeric vaccine candidate containing the pre- membrane and envelope genes of ZIKV substituted into the genome of ARPV. Herein, we evaluated the safety profile of AZ immunization using an anti-vector and coinfection studies.

Our results indicate: (i) ARPV and AZ remain incapable of replication in vertebrate cells during coinfection with ZIKV; (ii) Anti-ARPV immunity does not occur in immune-competent mice. We also further explored changes in immunogenicity and efficacy via dose-dependent, booster, and maternal antibody transfer studies. Our results indicate AZ is protective at  $\geq 10^{10}$  GC/mL in immune-competent mice, boosters increase immunogenicity, and a 3-dose series provides sterilizing immunity, and there is efficient transfer of maternal antibodies in protective quantities from immune-competent dams to pups. Overall, our data suggests chimeric vaccines using this insect-specific platform are a safe and effective method for the development of flavivirus vaccines.

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

**Xue Zhao**

Postdoc

## **Engineered yeast displaying specific norovirus-binding nanobodies for the concentration and detection of human norovirus in food matrix**

As the leading cause of foodborne illness worldwide, human noroviruses pose grave threats to public health and the global economy. The major drawback of existing detection methods was that they can only provide reliable and sensitive detection only if human noroviruses have been concentrated and purified from complex samples. Herein, our study aimed to genetically engineer food-grade yeast (*S. cerevisiae* EBY100) to display specific norovirus-binding single-domain antibodies (nanobodies) Nano-26 and Nano-85 on cell surface to facilitate concentration and purification of norovirus for easier detection. The bindings between nanobodies with yeast and norovirus virus-like particles (VLPs) were characterized and confirmed using confocal microscopy and flow cytometry analysis. The ability of our engineered yeasts on capturing norovirus VLPs was assessed using a BCA protein assay and the capture efficiency using EY-Nb-26 and EY-Nb-85 can reach up to 91.3% and 74.3%, respectively. Furthermore, we have utilized this approach to concentrate and detect norovirus in a real food matrix using a sandwich assay. A wide linear detection range ( $10 - 10^5$  pg/mL;  $R^2 = 0.9634$ ) was observed and the detection limit of norovirus VLPs on spiked spinach leaves was calculated as low as 0.86 pg/mL when EY-Nb-26 was utilized. Overall, our proposed yeasts could be a promising approach to concentrate and purify human noroviruses in food samples for easy detection, which allows to prevent the spread of foodborne virus in the food supply chain and reduce the risk of foodborne outbreaks.

**Keywords:** Norovirus; Genetically engineered yeast; Nanobody; Virus detection; Fresh produce

# Vector Biology, Vector-borne Diseases & Zoonotic Diseases

## Joanna Reinhold

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Faculty Mentor: Chloe Lahondere

Amphibian populations are in decline worldwide, largely due to pathogens that affect these animals. Ranaviruses infect amphibians, reptiles, and fish and have been the cause of mass die-offs in amphibian populations. Moreover, other parasites such as giant anuran trypanosomes can be pathogenic to their amphibian hosts. *Culex territans* is a mosquito species that feeds on amphibian hosts, primarily frogs, and is a suspected vector of parasites. Many mosquitoes in the *Culex* genus carry viruses, but no research has been done on this species' capacity to transmit viruses of veterinary or human significance. We hypothesized that *Cx. territans* is transmitting ranaviruses and trypanosomes to its amphibian hosts during blood-feeding. To test this hypothesis, we first tested field-caught mosquitoes and frogs for these pathogens at Mountain Lake Biological Station (Pembroke, VA). We focused on collecting *Cx. territans* mosquitoes as well as blood from two of their hosts, the green frog, *Lithobates clamitans*, and the bullfrog, *Lithobates catesbeianus*. Blood fed mosquitoes and frog blood were screened for ranaviruses and trypanosomes using cell culture overlay assays followed by DNA extraction and PCR. Both pathogens were found in several samples, indicating *Cx. territans* can uptake ranaviruses and trypanosomes and potentially transmit these pathogens to frogs. This research adds another critical piece to the puzzle of the epidemiology of anuran pathogens.