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

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

Oral Presentations

(in Duckpond)

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Semisynthetic Strategies To Access Novel Chloramphenicol Derivatives

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

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

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

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

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

Immunogenicity And Replication Capacity Of Recombinant Rotaviruses Expressing Norovirus Proteins

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

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

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

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Breakout Session #2: Computational Biology and Disease Modeling

Oral Presentations

(in Smithfield)

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A Synthesis Work on Climate Change and Coastal Ecosystem Health

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Abstract

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

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

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

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

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ABSTRACT:

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

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

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

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

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

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

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Abstract:

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

MATHEMATICAL FORMULATIONS FOR REPRESENTING HUMAN RISK RESPONSE IN EPIDEMIC MODELS

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

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

Oral Presentations

(in Cascades)

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Title

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

Authors

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Abstract

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

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

DISTRIBUTION OF THE BATS OF MALI

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Abstract

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

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

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

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

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

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

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

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

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

Entomopoxvirus Associated Polinton-like Viruses Provide Insight into Replicon Evolution

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

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

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

Oral Presentations

(in Duckpond)

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

ABSTRACT

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

MORTALITY IN MEXICO CITY DURING THE 1890 INFLUENZA EPIDEMIC

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

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

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

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

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

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

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

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

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Breakout Session #5: Immunology and Host-Pathogen Interactions

Oral Presentations

(in Smithfield)

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

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

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

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

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Abstract

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

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

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

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

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

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

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

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

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

Oral Presentations

(in Cascades)

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Chromosomal Inversions Differentiate Mosquitoes in *Culex Pipiens* Complex

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

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

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

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

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

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

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

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

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

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

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

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