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Discovering Novel Genes Utilized in the Insect Immune Response to West Nile Virus

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Insect-borne viruses, such as West Nile virus (WNV), pose a serious threat to public health worldwide. Because of the reliance on a mosquito for the transmission of WNV to humans, it is imperative that we understand and control the insect vector to reduce mosquito-to-human virus transmission. In this work, we utilize *Drosophila melanogaster*, an established model for immunity, to elucidate the antiviral immune response in an insect. Here, we use Kunjin virus (KUNV), a naturally attenuated subtype of WNV. We screened for novel components of insect immunity utilizing the *Drosophila* Genetic Reference Panel (DGRP), which is a panel of wild-type fly lines with sufficient genetic diversity to reveal genetic variants that contribute to increased susceptibility to KUNV infection. We determined the mortality rate for each fly line and used it for a subsequent Genome-Wide Association Study. Through this screen we identified single-nucleotide polymorphisms (SNPs) in the transcription factor binding site of dorsal (NF_KB) and in the coding region of insulin receptor and egfr (receptor for the MAPK pathway). We noted an enrichment of SNPs in gene ontology groups relating to the MAP kinase pathway (tak1 and basket) and to the JAK/STAT pathway (Su(var)2-10 and hopscotch). Using both in vitro and in vivo methods, we are validating the contribution of each of these candidate genes to the immune response to KUNV. We hypothesize an integration of the MAPK, insulin response, and JAK/STAT signaling pathways for the effective host response to KUNV infection. Future work will determine if these genes are also necessary for the mosquito response to KUNV, thus providing impetus to target these genes for the control of WNV and other mosquito-borne viruses.

Fecal Coliforms Increase in a Storm Drain Fed Pond After Rain Events

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Cannon Hill Park Pond (CHPP), Spokane, WA is a residential pond that has historically been maintained by the continuous input of potable water (˜14 million gallons/year, City of Spokane Water Quality Report Cannon Hill). In 2010, as part of the Lincoln Street Spokane Urban Runoff Greenways Ecosystem project, a vegetated bio-filtration cell (storm garden) was designed to capture and filter storm water and direct its flow to CHPP via a storm drain. It was meant to mitigate storm water and sanitary sewage overflow during storm events and contribute to CHPP water levels (estimated 315,000 gallons/typical year). While the City of Spokane has conducted some chemical analyses of CHPP, they have yet to conduct any fecal coliform (fc) testing. Our objectives were to compare fc levels in CHPP to levels recommended by the Washington State Department of Ecology (WA-DOE) and to determine if fc levels in creased with rain events due to the storm water input from the storm drain. To address these objectives, we used a membrane filtration method and cultured filters on mFC agar to identify fc bacteria. Samples were taken weekly for 15 weeks (10 non-rain events, 5 rain events) from three pond sites: directly in front of the storm drain, from the potable water spigot, and at an off-shore point >10m from the storm drain. Fc levels at the storm drain and at the off-shore site were significantly different ($p=0.0498$) and both exceeded WA-DOE recommended levels (10% samples exceed 400 fc/100 ml). Additionally, there was a significant increase in fc detected at the storm drain ($p=0.007$), but not at the off-shore site ($p=0.13$), after rain events. Fc were never detected in our potable water samples.

How Supplemental Iron Impacts the Antibacterial Effects of Manuka Honey

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Pathogenic bacteria have developed resistance to every antibiotic currently available, motivating scientists and medical professionals to find effective alternative treatments. Honey has captured the attention of researchers, due to its long history of effective medical use as an antibacterial. Manuka honey in particular, has proven to have strong antibacterial properties. In addition to hydrogen peroxide, low pH and high sugar content found in typical honeys, Manuka honey also contains methylglyoxyl from the *Leptospermum scoparium* flower nectar. The exact mechanism by which these Manuka honey components, and possibly others, work to kill bacteria and prevent resistance remains unknown. One hypothesis is that Manuka honey may interfere with iron acquisition. Bacteria require iron for many processes, including metabolism, gene expression, and enzyme activity; without it, they are unable to survive. Here, we test this hypothesis by determining if supplemental iron offsets the antibacterial action of Manuka honey. The minimum inhibitory concentrations for *Escherichia coli* and *Staphylococcus aureus* were 6% (n=8) and 3% (n=16) Manuka honey, respectively. When *E. coli* cultured with 6% Manuka honey was supplemented with ferrous sulfate (75, 100, 200, 300, 400, 500M), we observed growth that was significantly greater (p values = 0.0025, 0.0268, 0.0266, 0.0004, 0.0005, 0.0057) than the *E. coli* plus 6% Manuka honey alone. However, supplementation of *S. aureus* plus 3% Manuka honey with iron (25, 50, 100, 300, 400M) did not restore growth over the no iron control (p values = 0.6599, 0.7458, 0.0813, 0.0675, 0.1025). Instead, three of our iron treatments, 75, 200, and 500 M, resulted in significantly less growth (p= 0.0378, 0.0242, 0.0195) than *S. aureus* exposed to honey alone. We are further exploring the relationship between Manuka honeys antibacterial action and iron acquisition by repeating these supplementation experiments with *E. coli* mutants in glyoxylase (gloA) and iron uptake (fur).

T4SS Effector Protein Prediction: Feature Selection

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Bacterial pathogens have different methods for attacking host cells and one of them is by means of a secretion system. There are nine known secretion systems, and in this work, we consider type IV secretion systems (T4SS) composed of multiple proteins responsible for secreting effector proteins directly into eukaryotic host cells. It is very important for biologists to know how these effectors function, but first it is necessary to identify the effectors from among the entire genome of a bacterial pathogen. In recent years, some methods based on scoring and machine learning algorithms have been proposed to make this task easier by finding effector candidates to be tested in laboratories later. However, each work has proposed different sets of features. The main idea of our work was to find a set of optimal features to use in machine learning algorithms. For this purpose, we built a dataset of known effectors and non-effectors for four types of bacterial pathogens (*Legionella pneumophila*, *Coxiella burnetii*, *Brucella* spp, and *Bartonella* spp) as well as a set of all known features using a literature review. Then, by means of statistical tests and dimensional reduction methods, we selected a set of optimal features for T4SS effector prediction. Next we used known effectors for *Coxiella burnetti* to compare results using our feature set to a set of features proposed in a previous study. We showed that our optimal features give much better results than the feature set used in the previous work.

Hot Tales of T4's Transition from Host to Phage Metabolism

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When a lytic bacteriophage infects a host bacterium, it immediately begins transforming the host into a factory for phage production. The first steps in this process, once the phage is securely adsorbed to a complementary host, are the phage injecting its DNA and expression of early genes (over 100 of them for T4 –many of them host lethal,) tweaking the cell metabolism in a variety of largely little-understood ways that presumably make it more effective at phage production under at least some conditions. In the case of bacteriophage T4, the first alterations to its *Escherichia coli* host are cessation of the hosts maintenance and constitutive functions: host DNA synthesis and replication, cell division, transcription and translation of host genes and most kinds of macromolecular synthesis are all strongly inhibited within 1-3 minutes of infection. This behavior was originally characterized by introducing radioactively labeled substrates into T4-infected *E. coli*, and has been further explored across a variety of bacteriophage/host systems over the years using more modern "next-generation" techniques. Here, we review some of our early work in this field and discuss a number of our less-widely known studies of host-phage interactions using radioisotope labeling techniques, encouraging other labs to extend these areas of exploration to other genera of phages. While many biologists today have access to next-generation tools like RNAseq and metabolomics, the old standby of radiolabeling is still a reliable technique that offers unique capabilities for exploring many details of the phage infection process.

Characterization of *Helicobacter pylori* Cytotoxin Associated Gene Pathogenicity Island Putative Small Regulatory RNA Transcripts

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Helicobacter pylori is a human gastric bacterial pathogen responsible for gastric disease, including gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and MALT lymphoma. A specific *H. pylori* chromosomal DNA region, the cytotoxin associated gene Pathogenicity Island (cagPAI), is associated with more severe disease outcomes. The cagPAI encodes between 27-30 genes that produce a type IV secretion system pilus capable of injecting *H. pylori* factors into our gastric epithelial cells; injection of these factors into our cells results in signaling and morphological changes reminiscent of cancer precursor cells. We are interested in learning how genes within the cagPAI are regulated. In our recent study (Ta et al. 2012), we found two putative small regulatory RNA transcripts (sRNAs, RNA-III and RNA-XII) located within the cagPAI. sRNAs are known to regulate gene expression in *H. pylori* and other bacteria. Herein, we determined that sRNA-III and sRNA-XII are indeed sRNAs, being at least 188bp and 80bp, respectively. Additionally, we identified a third sRNA, sRNA-IX that is at least 203bp in length and antisense to cagY. We are still working to define the 3' end of these sRNA so they are likely longer than our initial prediction here. We located the 5'-ends of transcripts sRNA-III and sRNA-IX and have predicted their approximate promoter locations. Finally, we have used the online sRNA prediction resource, TargetRNA2 to predict which *H. pylori* genes may be regulated by sRNA-III, sRNA-IX, and sRNA-XII.

Smart Thermometers for Safer Milk: Promoting Milk Quality among Maasai Pastoralists of Tanzania

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Milk plays a central role in pastoralist diets but may be a source of foodborne pathogens and antibiotic resistance. To encourage hygienic milk-handling practices, a public health intervention was performed across four Maasai communities in northern Tanzania (N=105 households). Across villages, intervention strategies differed in how the health message was conveyed (narrative vs technical formats) and hygienic practices emphasized (milk boiling versus milk pasteurization). Intervention effectiveness was determined by relating thermometer adoption, knowledge, and attitudes to total bacterial counts of milk samples (N=989) across a three-week period for each site. Results indicate that health messages conveyed in narrative formats and emphasizing pasteurization produced the greatest rates of technological adoption, knowledge retention/spread, and decreases in bacterial counts. Interventions to encourage the normalization of hygienic milk-handling behaviors within pastoralist communities will benefit from introducing culturally-aware practices advertised in narrative formats.

Development of a Malignant Catarrhal Fever Vaccine

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Malignant catarrhal fever (MCF) is an often fatal disease caused by a group of herpesviruses. The MCF viruses are carried asymptomatically by adapted hosts, but can cause disease when transmitted to clinically susceptible animals, such as cattle and bison. Ovine herpesvirus 2 (OvHV-2), normally carried by sheep, is the most frequent cause of MCF worldwide. OvHV-2-induced MCF has a significant economic impact on American bison due to their high disease susceptibility. The development of an effective vaccine to MCF is our research top priority. Because OvHV-2 does not grow in cell culture, conventional methods to attenuate or modify viruses to be used as vaccines cannot be utilized, making vaccine development very challenging. Regardless, using new strategies and a rabbit model of infection, significant progress has been made in the last few years. As a potential MCF vaccine, we are currently working on the development of a non-pathogenic bovine herpesvirus-4 (BoHV-4) vector to express OvHV-2 glycoproteins known to induce immune responses capable of protecting animals from disease.

Host Cell-Free Culture Allows Identification of Factors Defining Niche Restriction and Uncovers Metabolic Capabilities of *Coxiella burnetii*

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Coxiella burnetii is a highly infectious zoonotic bacterium that causes Query (Q) fever in humans. Replication in the infected host occurs intracellularly in a phagolysosome like compartment called the *Coxiella* containing vacuole (CCV). In order for replication to take place in the CCV, *C. burnetii* requires specific physicochemical and nutritional conditions provided by the host. The obligate intracellular nature of this pathogen has historically prevented definitive analysis of *C. burnetii* requirements for replication. However, with the recent introduction of methods for targeted gene deletion and culture of *C. burnetii* under chemically defined host cell-free (axenic) conditions, metabolic capabilities of this pathogen can now be definitively deciphered. We show that *C. burnetii* replication under axenic conditions is dependent on acidic pH, low oxygen concentration, and presence of carbon dioxide. A nutritionally limited axenic medium only containing 17 amino acids support robust replication of *C. burnetii*. Of these 17 amino acids, glutamate was identified as a major gluconeogenic carbon source involved in biomass production. Phosphoenolpyruvate carboxykinase (PEPCK) represents the first committed step in gluconeogenesis. In order to determine the importance of glycolysis and gluconeogenesis in *C. burnetii* replication, deletion of pckA, encoding PEPCK, was performed and *C. burnetii* replication on glycolytic and gluconeogenic substrates determined. Although the *C. burnetii* genome does not encode a canonical hexokinase; a major enzyme allowing phosphorylation of glucose for entry into the glycolytic pathway, the *C. burnetii* pckA mutant showed that in addition to utilizing gluconeogenic substrates for replication, *C. burnetii* can also utilize glucose to generate biomass. Overall, this study sheds light on physicochemical and nutritional factors that dictate *C. burnetii* niche restriction, and demonstrates that *C. burnetii* can utilize glucose as a carbon source, for example under conditions of amino acid limitation.

Sequential hypertonic-hypotonic treatment of *Acinetobacter baumannii* biofilm enhances penetration of hydrophilic antibiotics

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Infections with bacterial biofilms are as serious challenges because biofilm communities are highly tolerant to antibiotics. This protection is attributed, in part, to a hydrated matrix that surrounds the bacterial community and that delays antibiotic diffusion. In this study, we evaluated whether it is possible to dehydrate a biofilm and then re-hydrate using distilled water with antibiotic as a means to increase antibiotic penetration and efficacy. *Acinetobacter baumannii* test-tube biofilms (24 h) were exposed to hypertonic concentrations of maltodextrin, sucrose or polyethylene glycol (PEG). These biofilms were then washed with distilled water containing 10 times the concentration of antibiotics needed to kill these bacteria in broth culture (50 μg/ml tobramycin, 300 μg/ml chloramphenicol, 20 μg/ml ciprofloxacin or 100 μg/ml erythromycin). Biofilms were then harvested and the number of viable cells (CFU) was determined. Depending on the treatment combination, this sequential strategy reduced the cell counts by 2-7 log ($P < 0.05$). Relative to tobramycin treatment alone, the efficacy of sequential treatment was evident for all osmotic compounds ($P < 0.05$). Sequential treatment with erythromycin or chloramphenicol was not different from using either antibiotic alone ($P \geq 0.2$). The difference in antibiotic performance is correlated with the degree of antibiotic hydrophilicity. Our findings support the clinical evaluation of sequential regimens of hyper- and hypotonic solutions as a means to enhance antibiotic efficacy against chronic biofilm infections.

The *Brucella abortus* Type IV secretion effector BspA is ubiquitinated inside host cells

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Brucella abortus is a Gram-negative bacterium that causes brucellosis, a prominent zoonotic disease worldwide that is responsible for sterility and abortions in animals and chronic infections in humans. *B. abortus* is an intracellular pathogen that uses a Type IV Secretion System (T4SS) to ensure its survival and growth in host cells such as macrophages, epithelial and reproductive cells, via delivery of T4SS effectors thought to modulate host functions. One of these T4SS effectors, BspA, is a *Brucella* secreted protein that localizes to the host endoplasmic reticulum (ER) when ectopically expressed, where it inhibits the secretion of host proteins. BspA is a small 22 kDa protein with high molecular weight banding patterns consistent with polyubiquitination profiles, when examined by western blot analysis, suggesting the protein is being modified. To test this hypothesis, a reciprocal immunoprecipitation of BspA and ubiquitin was performed on HeLa cells expressing 3xFLAG-BspA and HA-Ubiquitin, which demonstrated that BspA is ubiquitinated. Additionally, BspA's stability increased when cells were treated with the proteasome inhibitor MG132, suggesting that BspA is ubiquitinated and degraded by the proteasome. Lysine residues are common sites of ubiquitination, so mutations of the four lysine residues of BspA to alanines were generated by site directed mutagenesis to determine the location and role of ubiquitination of BspA. The ubiquitination status of all lysine mutants of BspA is currently being characterized. All BspA mutants still inhibited host protein secretion, suggesting that its ubiquitination, if abolished by these mutations, is not required for its effect on the host secretory pathway.

Isolation and Analysis of Cluster Q Phage Ein37 and F1 Phage Plumbus

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The student participants in our third cycle of the SEA-PHAGE project were again members of the interdisciplinary program Introduction to Natural Sciences, a year-long, full-time learning community with integrated instruction in biology, chemistry, and science process skills. During the fall quarter students collected and purified phages using enrichment cultures of *Mycobacterium smegmatis* mc^2 155 at 37 C. This year 23 phages were isolated from local soils, purified, and entered into the PhagesDB collection. Compared to prior years, we had a lower percentage of successful isolations. This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Successful DNA extractions were completed for most of the phages, although some difficulty in dealing with DNA stability was encountered. DNA quality and restriction enzyme experiments were used to select genomes for sequence analysis. Phages were also analyzed by transmission electron microscopy after negative staining with uranyl acetate. This resulted in clear images of all of the isolated phages, which appeared to be siphoviridae. The phages sequenced and analyzed were Ein37 and Plumbus. DNA from these phages was sequenced using the Illumina process at the Pittsburg Bacteriophage Institute. The sequence of Ein37 revealed a 53,748 bp linear double stranded DNA genome with a sticky fourteen bp 3' overhang and with a GC content of 67.4%. Analysis of the sequence of this phage confirmed that it was a siphoviridae in the Q cluster. BLASTn results showed that Ein37 had a 99% sequence identity with the other 7 members of cluster Q, indicating a high degree of similarity in this group, with almost half of the predicted genes being Phams unique to this cluster.The genome of Plumbus is a 54,468 bp dsDNA with a sticky ten bp 3' overhang and a GC content of 61.1%. BLASTn results indicated its closest relative was Cluster F1 phage Kimberlium. Both genomes were analyzed for potential protein coding open-reading frames using Glimmer and GeneMark, and protein functions were predicted by BLASTp and HHPred, as well as examining synteny with related phages. Preliminary results suggest the presence of 86 protein coding genes in Ein39 and 106 in Plumbus. We identified no tRNA or tmRNA genes in either phage. Further work is being conducted to identify and confirm all protein coding regions and to identify functions for predicted protein products.

Eliminating the bacterial endosymbiont *Wolbachia* from *Drosophila* species using a novel antibiotic delivery method

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Arthropods are common hosts for many pathogens and studying arthropod models can provide insight into pathogen-host interactions. In some cases, benign pathogens protect arthropod hosts from infection by more virulent pathogens. This phenomenon is observed in *Drosophila* species infected with the bacterial endosymbiont *Wolbachia*. *Wolbachia* is a vertically transmitted proteobacteria naturally infecting up to 70% of arthropods. Infection with this intracellular bacterium induces resistance to some RNA viruses by disturbing the insects innate RNA interference response. The *Drosophila* model is an excellent tool for furthering our understanding of pathogen-host interactions; however, when the pathogen in question is a virus, *Wolbachia* presence can confound results. Thus, eliminating *Wolbachia* from *Drosophila* allows for more accurate research of RNA virus infections. Curing *Drosophila* usually is done by rearing the flies on antibiotic-treated media; a process that can take months to produce *Wolbachia*-free flies that are ready for experimentation. Here we show that antibiotic microinjection into the *Drosophila* hemocoel successfully cleared infection; injected female flies produced *Wolbachia*-free offspring. Fecundity and sex ratios in the cured offspring were indistinguishable from untreated or mock-injected (buffer only) controls. This novel antibiotic delivery method greatly increases the speed (from several months to two weeks) and effectiveness (100% cure) of antibiotic treatment necessary to produce *Wolbachia*-free flies that are ready for experimentation.

Investigating Bacterial Communities Isolated from Mosquitoes Native to Spokane, WA

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Microbiomes within mosquito hosts can have a significant impact on transmission of arboviruses, such as the West Nile and Zika viruses. This ability to impact arbovirus transmission highlights the need to more thoroughly characterize microbial communities within mosquitoes. In this study, we sought to characterize the bacterial diversity in native mosquito populations from Spokane, WA, and to identify candidate bacterial genera to target in future studies. Samples of all mosquito life cycle stages were collected from both natural water sources and backyard gravid traps. Larvae, adults, and habitat water were spread onto ten different types of culture media following surface sterilization and homogenization in sterile water. From these original plates, morphologically distinct colonies (n=123) were selected and streaked for pure culture, of which 80 were putatively identified by PCR and sequencing targeting the 16S rRNA gene. The genera most commonly identified were *Aeromonas* (49%), *Pantoea* (6%), and *Pseudomonas* (5%). Of the *Aeromonas* species identified, the most common were *A. salmonicida* (n=22), *A. rivipollensis* (n=6), *A. veronii* (n=4), and *A. hydrophila* (n=3). All of these species save *A. rivipollensis* are known fish and/or human pathogens. *Aeromonas* species have a considerable impact on human health, yet their relationship to the mosquito microbiome is currently poorly detailed. Due to the high percentage of *Aeromonas* isolates discovered across a wide spectrum of sample types and location, and their importance as pathogens, this genus will be the target of future study to elucidate the role of bacteriophage in influencing bacterial communities within the mosquito host.

Whole proteome clustering reveals a set of proteins conserved across all recognized bacterial phyla

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The use of computational methods in comparative genomics has become central for studying early evolution and identifying essential genes. With the advent of high throughput sequencing techniques, more complete genomes have become available, and more networks can be constructed and explored. The combination of these networks with powerful network science approaches that allow the analysis of relationships within the networks gives us an unprecedented ability to understand more about organisms and the evolution of life. In this work, we applied Needleman Wunsch semi-global alignment algorithm using pClust software to cluster the complete proteomes from over 400 bacteria using a representational number from each of the 28 recognized bacterial phyla. We created a network of all the organisms and used network science approaches to find the essential genes. We identified 20 proteins that are shared among more than 99% of all the organisms and another 119 proteins that are shared among more than 94% of all the organisms. We postulate that these 139 proteins are evolutionarily conserved, as they are represented across the 28 phyla. Further, we suggest that these proteins are amongst the oldest proteins and are generally essential for life. Furthermore, our results determined there are several important organisms with a low percentage of GC content that are pathogens and Gram-negative and that also have strong connections with organisms from several other phyla. These similarities indicate that these organisms evolved from a common ancestor.

The Role of Intestinal Dysbiosis on CNS Inflammatory Demyelination in Non-Obese Diabetic Mice

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The gut microbiome plays a critical role in the regulation of central nervous system (CNS) demyelination in experimental models. Gut microbes influence the response of regulatory immune cell populations in the gut-associated lymphoid tissue (GALT); these immune cell populations confer protection in acute and chronic experimental autoimmune encephalomyelitis (EAE). Recent studies suggest that there is a bidirectional communication between the host and the microbiota. We hypothesized that the gut microbiota differs between the acute inflammatory and chronic progressive stages in a bi-phasic murine model of multiple sclerosis (MS) induced in non-obese diabetic (NOD) mice. Approximately 70% of NOD mice develop a severe form of EAE while the remaining develop no or mild symptoms of disease. We compared the gut microbiota of NOD mice with either mild or severe disease states to healthy control mice. Moreover, we evaluated whether or not the outcome of progressive stages of EAE in the NOD model was modified by the administration of a broad-spectrum antibiotic cocktail. To assess the functionality of the differing microbiomes between disease severity states, we generated putative annotations on bacterial operational taxonomic units (OTUs) using PICRUSt. To determine whether different autoimmune diseases affect the microbiome similarly we extended the comparisons to severe EAE versus diabetic mice. Our findings support the hypothesis that there are reciprocal effects between experimental autoimmune diseases and modification of the microbiome; early therapeutic interventions that target the gut microbiome could potentially limit disease progression.

Controlled activity of the invasion-associated injectisome reveals its intracellular role in defining the cytosolic *Salmonella* population

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The *Salmonella* invasion-associated type III secretion system (T3SS1) is an essential virulence factor required for entry into non-phagocytic cells and consequent uptake into the *Salmonella*-containing vacuole (SCV). While *Salmonella* is typically regarded as a vacuolar pathogen, a subset of bacteria escape from the SCV in epithelial cells and eventually hyper-replicate in the cytosol. T3SS1 is rapidly down-regulated following bacterial entry into mammalian cells, but cytosolic *Salmonella* are T3SS1-induced, suggesting resurgent activity of T3SS1 in this population. T3SS1 mutants are invasion-defective, which has limited assessment of the T3SS1-dependence of such intracellular activities. Here we define the post-internalization contribution of T3SS1 to the infectious cycle of *Salmonella* by tagging the T3SS1-energizing ATPase, InvC, at the C-terminus with peptides that are recognized by bacterial C-terminal-specific proteases, dramatically increasing its turnover. The power of this approach is that the T3SS will be energetically "dead", even if the injectisome is assembled. Using an anhydrotetracycline (ATc)-inducible promoter, these bacterial strains were proficient for invasion in the presence of ATc, but underwent rapid and sustained inactivation of T3SS1 activity upon ATc withdrawal. This allowed us to directly implicate T3SS1 activity in cytosolic colonization and bacterial egress. We further identified two T3SS1-delivered effectors, SopB and SipA, as being required for efficient colonization of the epithelial cell cytosol. Overall, our data supports a multi-faceted, post-invasion role for T3SS1 and its effectors in defining the cytosolic population of intracellular *Salmonella*.

Alphaherpesvirus entry into cells by a conserved proteasome-dependent mechanism

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Alphaherpesviruses are ubiquitous and are host-adapted to many mammalian species. This herpesvirus subfamily has a propensity to infect mucosal epithelial cells as a portal of entry into the host. The peripheral nervous system serves as the site of alphaherpesvirus latency. Thus, alphaherpesviral entry includes conserved cellular mechanisms. Initial infection by herpes simplex virus 1 (HSV-1) requires host cell 20S proteasome activity. The virion tegument component, infected cell protein 0 (ICP0) is a viral E3 ligase and directs efficient trafficking of entering HSV-1 capsids to the host cell nuclear periphery. Notably, ICP0 is conserved among alphaherpesviruses and functions similarly in stimulating efficient lytic replication. However, whether the degradative activity of the proteasome drives the entry of other alphaherpesviruses is unknown. We investigated the ability of proteasome inhibitors such as MG132 to inhibit the entry or infectivity of three additional alphaherpesviruses: pseudorabies virus (PRV) a swine pathogen, bovine herpesvirus 1 (BoHV-1) a key component of the respiratory disease complex in cattle, and HSV-2, an important cause of sexually transmitted disease in humans. Using beta-galactosidase reporter entry assays or plaque assays of infectivity, PRV entry into porcine kidney (PK-15) cells, BoHV-1 entry into Madin-Darby bovine kidney cells, and HSV-2 infectivity into Vero cells were all blocked by proteasome inhibitors in a concentration-dependent manner. Importantly, inhibition occurred at non-cytotoxic concentrations of drug as determined by LDH cytotoxicity assay. The results suggest that proteasome-mediated degradation of a viral or host factor is a conserved feature of entry among alphaherpesviruses. Additional work is needed to define the precise, proteasome-dependent step in the entry of each virus and whether ICP0 plays a similar role in the entry of PRV, BoHV-1 and HSV-2.

Autoinducer-2 Quorum Sensing Contributes to Regulation of Microcin PDI in *Escherichia coli*

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Quorum sensing (QS) is a concentration-dependent signaling system by which a population of bacteria can alter and synchronize activities (gene expression, motility, etc.) in the presence of a threshold concentration of a signaling molecule. In *Escherichia coli* the QS signal molecule, autoinducer-2 (AI-2), reaches the maximum concentration during mid-to-late growth phase after which it quickly degrades during stationary phase. This pattern of AI-2 concentration coincides with the up- then down-regulation of the recently described microcin PDI (mccPDI) effector protein (McpM). A prototypical mccPDI-expressing strain of *E. coli* (*E. coli*-25) was used to generate ΔluxS, ΔlsrACDBFG (lsrH), and ΔlsrR mutants, which are responsible for AI-2 production, transportation, and AI-2 transport regulation. Trans-complementation, RT-qPCR, and western blot assays were used to detect changes of microcin expression and synthesis under co-culture and monoculture conditions in M9 minimal media. When deficient in QS the PDI producer strain was defective for inhibition of susceptible bacteria. Compared to the wildtype strain, the AI-2-deficient strain (ΔluxS) and uptake negative strain (ΔlsrH) were <1000-fold less inhibitory to susceptible bacteria. The AI-2-deficient mutant was mostly restored by in trans complementation of luxS. RT-qPCR and western blot results for the AI-2 deficient *E. coli*-25 showed a 5-fold reduction in mcpM transcription with an average of 2-h delay in McpM synthesis. This is the first evidence that class IIa microcin regulation can be linked to quorum sensing in a Gram-negative bacterium.

Role of the Nipah Virus Fusion Protein and Glycoprotein on Innate Immune Responses

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Nipah Virus (NiV) is an RNA virus with a 40-90% mortality rate. Since there are no approved vaccines or cures for NiV we are working to understand the immune response to infection, which could aid in antiviral approaches to NiV. By invoking unique mechanisms to evade the host immune response, NiV can increase its infectivity potential, replicate to higher levels, and spread from one host to another. The innate immune response is the first line of defense for infected hosts to combat viral infection. Thus, viruses increase their infectivity through mechanisms that inhibit the innate immune response. Two proteins of NiV have been shown to inhibit immune responses and we investigated the potential role of two other NiV proteins, the fusion protein (NiV-F) and glycoprotein (NiV-G), in modulating the innate immune response of infected host cells. We hypothesized that NiV-F and NiV-G would modulate innate immune responses of the host cell through inhibition of intracellular proteins, RIG-I and MDA5, that are known to initiate innate immune response signaling upon recognition of viral RNA. Through an in vitro luciferase assay we quantified innate immune responses in the presence and absence of NiV-F and NiV-G. Results indicated that there was a statistically significant decrease in innate immune response when NiV-F and NiV-G were present. There was also a decrease in RIG-I and MDA5 expression in the presence of NiV-F or NiV-G. Thus, NiV-F and NiV-G may degrade or inhibit the on-demand expression of the intracellular proteins by an unknown mechanism. This, in turn, would result in impairment to initiate a strong innate immune response. These results suggest that NiV utilizes NiV-F and NiV-G to evade innate immune responses. Current work is being done in which primary cells are chemically stimulated to induce IRF-3 mediated innate immunity and in the presence of NiV proteins we expect activity of this pathway to decrease. In the future, we would like to investigate further the mechanism of how NiV-F and NiV-G are mediating the inhibition of the innate immune responses. NiV-F and NiV-G proteins may be a cornerstone of the viruss ability to evade the host innate immune response and targeting these proteins in future therapeutics could aid in proper host defense against NiV infection.

Creating a Genetic Tool for Visualization of H-NS *in vivo*

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H-NS is a nucleoid associated protein of enterobacteria that silences many genes including the virulent Locus of Enterocyte Effacement (LEE) genes of Enteropathogenic *E. coli.* (EPEC). I have fused H-NS to mEo3.2, a photoactivatable protein that will allow H-NS to be visualized *in vivo* using Photo-activated Localization Microscopy (PALM). Beta-galactosidase assays indicated that this construct was functional in that it successfully silenced LEE-5 in a delta hns strain with a LEE5-lacZ fusion (KM4115) to a similar degree as a wild type control. Other controls of the assay included just KM4115, the pACYC184 vector without my construct in KM4115, hns transformed back into KM4115 on a pBR322 vector, and the empty pBR322 vector in KM4115. Preliminary images of the construct suggest that H-NS forms two distinct aggregates in the cell. This fusion can further be used to answer future research questions such as what happens to H-NS localization in the cell when an anti silencer of the protein, like Ler, is induced?

Pseudorabiesvirus, a Swine Alphaherpesvirus, Enters Cells via a Low pH-Dependent Endocytic Pathway

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The alphaherpesvirus pseudorabies virus (PRV) is the causative agent of pseudorabies, a disease of great economic and welfare importance in swine and dead-end hosts such as cats and dogs. Like the prototypical alphaherpesvirus herpes simplex virus 1 (HSV-1), PRV establishes primary infection at epithelial surfaces, followed by lifelong latent persistence in peripheral nervous system neurons and periodic reactivations at the initial site of infection. PRV demonstrates an ability to infect multiple species and cell lines, especially under experimental conditions. Other alphaherpesviruses including HSV-1 utilize multiple entry pathways in a cell-specific manner, which may contribute to their broad tropism. We demonstrate that PRV enters the porcine epithelial cell line PK-15 via low pH-dependent endocytosis. PRV entry into PK-15 cells was inhibited by ammonium chloride, a weak base that accumulates in lysosomes and raises the normally-acidic pH. PRV entry was similarly inhibited when endosomal acidification was blocked by the carboxylic ionophore monensin. Inhibition of entry occurred at non-cytotoxic levels of lysosomotropic agent, and in an agent concentration-dependent manner. Ammonium chloride and monensin exerted their effects at an early stage of infection, likely post-internalization and prior to viral maturation and processing. Inactivation of virions by acid pretreatment is a hallmark of viruses that utilize a low pH-activated entry pathway. Pretreatment of PRV virions with a mildly acidic pH of 5.0 to 6.0 inhibited viral entry into PK-15 cells, consistent with the low pH entry activity of PRV.

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perABC-mediated niche adaptation in EPEC

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EPEC (enteropathogenic *E. coli*) is a well-studied pathogen that causes infantile diarrhea throughout the developing world. EPEC infects the small intestine, forming attaching and effacing (A/E) lesions. The protein PerC, encoded by the per (plasmid-encoded regulator) operon, is an important stimulator of the LEE (locus of enterocyte effacement), a pathogenicity island necessary for infection. Yet, the upstream regulation of per is not well characterized in EPEC. Here, we create a genetic construct for future identification of factors in the distal small intestine that increase expression from the per operon. We have evidence of per regulation by the stringent response, and will use the construct to test this further.

Bovine herpes virus 1 enters via a low pH-dependent endocytic pathway in a cell type-specific manner

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Bovine herpesvirus (BoHV-1) is an epitheliotropic and neurotropic pathogen that poses a significant challenge to health and welfare in the cattle industry. Because BoHV-1 establishes life-long latency, effective disease prevention necessitates control of initial infection, yet the entry mechanisms utilized by BoHV-1 are undefined. Here we present a model for BoHV-1 entry by endocytosis and exposure to low intraendosomal pH. Treatment of Madin Darby Bovine Kidney (MDBK) epithelial cells with the lysosomotropic agents ammonium chloride or monensin, both of which elevate the low pH of organelles and thereby prevent endosomal acidification, inhibited BoHV-1 entry in a concentration-dependent manner as measured by a reporter assay. The kinetics of BoHV-1 entry via this pathway was rapid. 50% of infectious particles entered MDBK cells by 5 minutes post-infection. Additionally, the inhibitory effect of ammonium chloride was most pronounced when this agent was applied in the first hours of the viral infection period, suggesting that lysosomotropic agents specifically target viral entry. Treatment of African green monkey (Vero) cells with ammonium chloride did not significantly inhibit BoHV-1 entry, suggesting a pH neutral entry route in these cells. Interestingly, the related alphaherpesvirus HSV enters Vero cells via direct penetration, independent of low endosomal pH. Inactivation of virions by pretreatment with mildly acidic pH is a hallmark characteristic of viruses that utilize a low pH-activated entry pathway. When BoHV-1 viral particles were exposed to pH 5.0 prior to cellular inoculation, viral infectivity, as measured by plaque formation on MDBK cells, was markedly reduced. Together, these results support a model of BoHV-1 infection in which low endosomal pH is a critical, cell-specific host trigger for fusion of the viral envelope with an endosomal membrane, and necessary for successful infection of the target cell.

Environmental MRSP Surveillance in Primary Care Small Animal Hospitals: A Cross-Sectional Multi-Clinic Study

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Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a rapidly emerging, worldwide, veterinary and public health concern. Various types of infections are associated with MRSP and environmental contamination and persistence is a concern surrounding exposure/transmission. Our hypothesis is that the proportion of MRSP isolates collected from human hand contact surfaces will be significantly higher than the proportion collected from animal contact surfaces in small animal primary care veterinary clinics. We expect that cleaning/disinfection efforts in veterinary settings are focused on animal contact surfaces while human hand contact surfaces are neglected.

Samples were collected from eleven clinics. Surfaces were wiped with electrostatic cloths and incubated (35°C, 24 hr) in Tryptic Soy Broth (Hardy Diagnostics, Santa Maria, CA) (90mL, 2.5% NaCl). Next samples were struck to Mannitol Salt Agar (Hardy Diagnostics, Santa Maria, CA) (2ug/mL oxacillin) and incubated (35°C, 24-48 hr). Yellow colonies were sub-cultured to Columbia Blood Agar and incubated (35°C, 18-24 hr). β-hemolytic colonies were confirmed by the Washington Animal Disease and Diagnostic Laboratory. A survey was also delivered to each clinic about hospital infection control practices.

Overall prevalence of MRSP was found to be 12.6% (all clinics and surfaces). Hand contact surfaces ranged from 0-21% (med 6%, avg 7%), and animal contact surfaces ranged from 0-30% (med 0%, avg 6%) among all hospitals. The within-hospital difference between hand vs. animal contact surface prevalence ranged from 0-15% (med 3% avg 4%). No significant difference ($\alpha = 0.05$) between levels of contamination on human vs. animal contact surfaces was detected. ($p > 0.05$). The survey revealed that animal contact surfaces are cleaned significantly more frequently that hand contact surfaces ($p < 0.00001$).

A significant difference in the frequency of MRSP contamination between hand vs. animal contact surfaces was not detected, but was it was recovered at a significantly high frequency suggesting a serious concern. The human contact surfaces in this study were unlikely to come into contact with animals, therefore, contamination likely occurred via human hands. Hand hygiene in a veterinary setting should be improved. The study provides valuable information for further study regarding infection control practices in veterinary clinics.

The iron-dependent transcriptional repressor YtgR regulates tryptophan biosynthesis in *Chlamydia trachomatis*

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Chlamydia is strictly dependent on iron for proper development, yet the mechanisms of iron homeostasis remain poorly understood. We have shown previously that the transcriptional repressor YtgR regulates its own promoter in an iron-dependent manner in vitro. To better understand the immediate response to iron-limitation in *Chlamydia*, we performed an RNA-Sequencing experiment on *C. trachomatis*-infected HeLa cells treated short-term with the iron chelator 2,2-Bipyridyl (Bpdl). This data revealed that following brief iron starvation, the expression of the tryptophan (Trp) synthase subunits (trpBA) is highly upregulated while expression of the Trp-dependent repressor (trpR) residing within the same operon remains stagnant throughout treatment. Thus, we hypothesized the existence of a secondary iron-responsive mode of transcriptional regulation of the trpRBA operon mediated by YtgR. Using a two-plasmid reporter assay in *E. coli* that reports on YtgR repressor activity, we have demonstrated that YtgR is represses reporter gene expression from a putative promoter element upstream of trpBA, but not from the canonical trpR promoter. By mapping the 5'-ends of trpRBA transcripts generated under iron starved conditions, we implicate a novel transcriptional start site in the up-regulation of trpBA expression. Additionally, we show that protein expression of YtgR is dependent on the availability of tryptophan, and this dependency is likely the consequence of a motif of three sequential tryptophan codons in the coding sequence upstream of YtgR. This suggests that YtgR regulation of trpBA may function as a regulatory feedback mechanism to synthesize Trp in order to translate YtgR in times of stress. Collectively the data implicates YtgR as a critical transcriptional regulator in *Chlamydia* integrating the responses to the depletion of both iron and tryptophan.

Pulmonary tissue-resident CD4+ T cells contribute to early control of virulent *Francisella tularensis* infection

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Pulmonary infection with the highly virulent intracellular bacterium, *Francisella tularensis* (Ftt) results in an acute, lethal disease called tularemia. Depletion of CD4+ T cells in Ftt immune animals results in death within 5 days of infection, underscoring the importance of a rapid T response. We hypothesized that optimal protection against Ftt would require T cells poised to respond at the site of infection rather than those circulating through this compartment. We analyzed three pools of anatomically distinct lung T cells- airway, circulating, and tissue resident- in mice vaccinated with *F. tularensis* Live Vaccine Strain (LVS). Vaccination increases the number of tissue resident, poly-functional effector CD4+ T cells while nave and immune mice had similar numbers of circulating CD4+ T cells. Upon challenge with Ftt, there is an early expansion of poly-functional, tissue resident CD4+ T cells while the increase of circulating CD4+ T cells is delayed. We isolated the contribution of resident T cells in the control of Ftt infection by inhibiting T cell trafficking from the circulation into the lung parenchyma using FTY720. Mice treated with FTY720 had similar bacterial burdens as vehicle-treated mice suggesting resident CD4+ T cells are sufficient for early control of Ftt replication within the pulmonary compartment. Together, these data suggest that expansion and maintenance of a pool of polyfunctional, tissue resident CD4+ T cells, and not circulating CD4+ T cells, are critical for protective immunity against Ftt. Thus, our results provide important insights into the nature of the protective CD4+ T cell response that must be provoked by future vaccine candidates.

The proteasome inhibitor bortezomib blocks herpes simplex virus infection

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Herpes simplex virus (HSV) is a lifelong pathogen that causes an array of diseases, ranging from cold sores, sexually transmitted genital lesions, and ocular disease to rare but severe cases of encephalitis. While most infections are asymptomatic, immune-compromised and neonatal patients are particularly vulnerable to the virus. The antiviral drug acyclovir is the most common treatment of HSV infection. Acyclovir is a nucleoside analog that is inserted into the nascent viral DNA chains causing termination of HSV replication. HSV strains resistant to acyclovir arise readily due to acyclovir's targeting of a virus-specific process. Acyclovir-resistant HSV can be devastating to vulnerable populations. New options for antiviral treatment are needed. We propose that the proteasome inhibitor bortezomib is a novel therapeutic to combat HSV infection. HSV requires the proteasome to enter the nucleus for replication. Therefore, we hypothesized that using the proteasome as a drug target would limit the development of resistance, as there would be no direct pressure on viral components. Bortezomib is an FDA-approved anti-cancer agent for treatment of multiple myeloma. Preliminary results suggest that it is effective against HSV infection at non-cytotoxic concentrations. We aim to determine if the drug is effective against acyclovir-resistant HSV, as well as wild-type and clinically-isolated strains. To elucidate the mechanism of action of bortezomib, time of addition and fluorescence microscopy studies revealed that bortezomib acts on a step early in infection including halting transport of entering HSV nucleocapsids to the host cell nuclear periphery. Current and future studies aim to determine the identity of virus or host factors targeted for degradation during HSV entry. Developing bortezomib as an effective, new antiviral strategy has the potential to replace or supplement existing treatments.

The *Yersinia pestis* carbon storage regulator (CsrA) regulates in vitro biofilm production and a transmission competent biofilm in *Xenopsylla cheopis* fleas by responding to specific nutrients

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Yersinia pestis, the bacterial agent of plague, is acquired by fleas through blood-feeding on an infected host. Following expression of adaptive traits the bacterial pathogen multiplies within the flea midgut to produce a thick coherent biofilm. Successive development of this biofilm in the flea foregut proventriculus leads to a biofilm-mediated blockage that results in transmission of the pathogen during subsequent blood feeding. How *Y. pestis* adapts to the physicochemical status of the flea gut to facilitate blockage-mediated transmission is unknown. However, the carbon storage regulator protein, CsrA, a highly conserved protein in many bacterial species, which temporally coordinates various physiological adaptations to changing nutritional environments, including biofilm development, is highly transcribed in *Y. pestis* during blockage. In *Y. pestis* it was previously shown using an indirect in vitro biofilm assay that CsrA positively regulated biofilm production. Therefore, we hypothesize that CsrA is required for biofilm-mediated blockage transmission of fleas. To test this hypothesis we first compared growth between the wildtype, ΔcsrA mutant, and ΔcsrA mutants complemented in trans or in-cis. We demonstrate that the ΔcsrA mutant grows slower and the growth phenotype can only be restored by in cis complementation. Next, we used Congo red binding assays to directly measure biofilm formation irrespective of growth to test the ability of the ΔcsrA mutant to produce biofilm. This assay reveals that csrA is necessary for productive biofilm formation in response to particular nutrients. Finally, the ΔcsrA mutant was tested for its ability to infect the oriental rat flea, *Xenopsylla cheopis*. Fleas infected with the ΔcsrA mutant were shown to block at significantly reduced rates versus the wildtype. Collectively our data suggests that CsrA regulates a transmission competent biofilm infection in the flea in response to specific nutrients.

Brucella effector BspB targets secretory traffic to promote intracellular bacterial replication

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Many intracellular pathogens exploit host secretory trafficking to support their intracellular cycle, but knowledge of these pathogenic processes is limited. The bacterium *Brucella abortus* uses a Type IV secretion system (VirB T4SS) to generate a replication-permissive *Brucella*-containing vacuole (rBCV) derived from the host endoplasmic reticulum (ER), a process that requires host early secretory trafficking. Here we show that BspB, a VirB effector, contributes to rBCV biogenesis and *Brucella* replication by interacting with the Conserved Oligomeric Golgi (COG) tethering complex, a major coordinator of Golgi vesicular trafficking, remodeling membrane traffic at the Golgi interface with the intermediate compartment and redirecting Golgi-derived vesicles to the BCV. Altogether, these findings demonstrate that *Brucella* modulates COG-dependent trafficking via delivery of a T4SS effector to promote rBCV biogenesis and intracellular proliferation, providing mechanistic insight into how bacterial exploitation of host secretory functions promotes pathogenesis.

Comparative Analysis of Select DNA Replication Genes from the *Diachasmimorpha longicaudata* entomopoxvirus

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The *Diachasmimorpha longicaudata* entomopoxvirus (DlEPV) is an insect-specific poxvirus found the in the poison glands of *D. longicaudata*, a parasitoid of tephritid fruit flies. DlEPV undergoes morphogenesis in the poison gland accessory filaments of the parasitoid braconid wasp (*D. longicaudata*). The virus is deposited into larval hosts (*Anastrepha suspensa*) during oviposition. DlEPV replicates in and buds from the host's haemocytes, putatively disrupting the encapsulation response to the wasp egg. The DlEPV virion is 250-300 nm in diameter, with a dumbbell-shaped core. It has a unipartite, double-stranded DNA genome that is estimated at 290-300 kb in length. An assortment of DlEPV genes has been sequenced and each gene exhibits significant similarity to EPV homologs. Nevertheless, DlEPV remains an unclassified member of the Entomopoxvirinae. Thus, our goal is to further elucidate DlEPV's taxonomic position. To this end, poison glands were dissected from *D. longicaudata* females and DlEPV virions were isolated using sucrose gradient ultracentrifugation. Virion DNA was purified and sequenced using Illumina. The resulting reads assembled into contigs (1,000-30,000 bp) in CLC Workbench. A full genome did not assemble. From these contigs, homologs ($E \leq 3.00E\text{-}15$) of four EPV genes involved in DNA replication were identified (helicase, primase, polymerase, topoisomerase). To further clarify homology, multiple sequence alignments (MSA) were performed (Clustal Omega) and phylogenetic trees were constructed (Geneious R9). Phylogenetic analysis confirms that DlEPV is an EPV but indicates that DlEPV does not belong in the Alphaentomopoxvirus or Betaentomopoxvirus genus. Given that sequence data for accepted Gammaentomopoxvirus members are unavailable, we suggest that DlEPV should be classified into the Gammaentomopoxvirus genus, or be made the type species of a new genus.

Repeated Motif Potentially Related with Plasticity of the Aaap Locus in *Anaplasma*

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The *Anaplasma* appendage-associated protein, Aaap, is closely associated with inclusion appendages formed when *Anaplasma marginale* infects mature erythrocytes. These inclusion appendages appear like tails next to the parasitophorous vacuole, and contain highly structured actin bundles. Aaap is encoded in a highly dynamic locus that contains additional genes with similarity to Aaap, known as alps for Aaap-like proteins. These proteins are characterized by repeat sequences. Alignment of these protein sequences from different species of Anaplasma reveals six similar repeating motifs. Examination of all 56,772,018 protein sequences in the GenBank NR database shows that this special repeating feature is only shared among certain species of *Anaplasma*, which is likely to account for the formation of inclusion appendages in erythrocytes infected by these *Anaplasma* species. In this work, first we discover which species of *Anaplasma* have repetitive motif patterns of high similarity. Second, we analyze the repetition from a statistical perspective. Third, we further explore how this repetitive pattern influences the aaap locus.

Dropping Like Flies: Innate Immune Responses of *Drosophila melanogaster* During West Nile virus Infection

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Insects frequently transmit vector-borne viruses, including the flaviviruses West Nile virus (WNV), dengue virus (DENV), and Zika virus (ZIKV). Although there are preventative measures in place to minimize the spread of flaviviruses, there is no approved vaccine or treatment post-exposure. Because of this unmet need in public health, we seek to understand how insects tolerate WNV infections, which could assist in the development of an effective therapeutic. Here, we utilize *Drosophila melanogaster* as a model organism to understand immunity in an insect. In our study, we use Kunjin virus (KUNV), a naturally attenuated subtype of WNV to probe an immune response in *Drosophila* and determine which components of the immune response are critical in the antiviral response. We infected flies carrying mutations in genes required for immunity, with emphasis on genes pertaining to the JAK/STAT pathway and Insulin Response. We measured survival and viral load in the flies to determine if these genes are important for a full immune response to KUNV. Based on preliminary studies, we have shown that genes within the JAK/STAT pathway impact fly survival. We have determined that hosts with mutated upaired, STAT92E, vir-1, and Insulin Receptor genes exhibit increased mortality and viral load when exposed to the KUNV due to their importance in the antiviral innate immune response. This research will help us understand which insect genes are responsible for the immune response to flaviviruses. Ultimately, we can use this research to direct future studies regarding the mosquito immune response to flaviviruses.

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