EPI RESEARCH DAY BOOK OF ABSTRACTS

DEMYSTIFYING DISEASES
TO CREATE EFFECTIVE CURES

FEBRUARY 2016

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Letter from the Director

Welcome to the ninth annual EPI Research Day! As you look through the abstracts in this book, and view the correlating posters, you should get a feel for the wide range of emerging pathogens-related research conducted by EPI members and collaborators. We are particularly pleased to welcome investigators from outside of UF, including the Florida Department of Health, and other collaborating universities.

This year we have the honor of introducing you to two outstanding investigators visiting us to give speeches during our afternoon session.

Dr. Adel Mahmoud, professor of molecular biology and public policy at Princeton University, is a leading expert on vaccine development and infectious diseases in the developing world. Previously, Dr. Mahmoud served as president of Merck Vaccines, where he led the effort to develop a measles, mumps, rubella, and varicella vaccine, a shingles vaccine, and others. He is joined by Dr. Ilaria Capua, the former director of the Division of Comparative Biomedical Sciences at the Istituto Zooprofilattico Sperimentale delle Venezie in Padova, Italy. Dr. Capua trained as a veterinarian and has a research background in influenza viruses and viral zoonoses. Since February 2013 she has been a member of the Italian Parliament. A professor of animal sciences, Dr. Capua will soon join the University of Florida as the director of the Center of Excellence in One Health Research.

Please visit our website, www.epi.ufl.edu, to join our list-serves, and to keep up with our news, events and seminars throughout the year. And thanks for coming!

J. Glenn Morris, Jr. M.D., M.P.H. & T.M. EPI Director and Professor of Medicine

Schedule of Events

9:00 AM - 10:00 AM Registration, Breakfast, and Poster

Setup

CGRC 1st, 3rd, and 4th floors

10:00 AM - 1:00 PM Poster Session

Presenters, please stand by your

posters

12:00 PM - 12:45 PM Lunch

CGRC 1st floor Lobby

12:45 PM - 1:00 PM Keynote Assembly

CGRC Auditorium 101

1:00 PM - 1:10 PM Welcome

Dr. David Norton, VP of Research

Introductions

Dr. J. Glenn Morris, Director, EPI

1:10 PM - 3:15 PM Keynote Speeches

3:15 PM - 4:00 PM Poster Removal

Keynote Speakers

(1:10-2:10)

Dr. Adel A. F. Mahmoud, M.D., Ph.D.

The Woodrow Wilson School of Public Health and International Affairs The Department of Molecular Biology Princeton University

"Vaccine's Make a Difference, But?"

(2:10-3:10)

Dr. Ilaria Capua, D.V.M., Ph.D.

Incoming Director of the Center of Excellence in One Health Research Professor of Animal Sciences, IFAS

"Harnessing Research Potential from Emerging Viruses – 15 Years with the Flu"

01. ALLOSTERIC INHIBITORS OF ENTEROVIRUS POLYMERASES

<u>Catherine H. Schein</u> - Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Foundation for Applied Molecular Evolution (FfAME)

Enteroviruses (EV), include poliovirus, Coxsackie viruses, Rhinoviruses and many other human pathogens that can cause severe infections, especially in immune suppressed individuals. There are millions of infections with non-Polio EV in the US every year, all of which cause at least the loss of 1-3 working days. More severe infections can lead to life threatening infections, including pneumonia and paralysis. Chronic infections can lead to dilated cardiomyopathy and other disorders. There are currently no clinically relevant inhibitors of EV, or vaccines against non-Polio EV.

For inhibitor design, a reaction that is specific to EV was chosen. EV RNA-polymerases.begin replication by uridylylating (i.e., ransferring a UMP moiety to) a 22 amino acid peptide, VPg, as the first step in their replication. VPgpUpU, found free in infected cells, serves as the primer for RNA elongation (1). We recently showed that four diverse polymerases (3Dpol), from EV-species A-C uridylylate 5 VPgs that varied by up to 60% of their residues(2).All 3Dpol also uridylylated a consensus VPg designed to represent the physical chemical properties of 31 different EV-VPgs. Thus the residues and mechanism required for uridylylation must be similar in all EV, indicating that specific inhibitors of this reaction could be promising wide-spectrum therapeutics.

A large library of compounds were docked with PyRX to the surface binding site for VPg on 3Dpol seen in co-crystal structures and verified with mutation experiments. Compounds with good docking scores and specificity for the VPg binding site were tested for their ability to alter VPg uridylylation and subsequent formation of

VPgpolyU in a PAGE-based assay (2). Inhibition of the polymerase was not strictly linear with docking score, but several compounds with the highest predicted affinity (BE/MW) and specificity for the surface site were the most efficient inhibitors of uridylylation. At low [VPg], some of the weaker inhibitors activated the polymerase (3). These results indicate that the binding site for VPg is also an allosteric site for controlling the activity of the polymerase. The inhibitors identified here could be the basis for novel therapies.

02. ASSESSMENT OF THE EFFECT OF DIFFERENT FATS ON PATHOGEN SURVIVAL IN COOKIE DOUGH

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College of Agricultural and Life Sciences, University of Florida; Kwang
Cheol Jeong - Department of Animal Sciences, College of Agricultural
and Life Sciences, University of Florida; Soohyoun Ahn - Department
of Food Science and Human Nutrition, College of Agricultural and Life
Sciences, University of Florida

Cookie dough is recognized as a potential vehicle for Salmonella for using egg as one of its ingredients. In 2009, commercial raw cookie dough was also reported as a novel vehicle for E. coli O157:H7 transition. Rapid detection methods for pathogens in cookie dough are in critical need to prevent foodborne outbreak and ensure food safety. The goal of this study is to compare rapid detection essays, including multiplex PCR and real-time PCR, to standard culture method for their sensitivity in detecting Salmonella and E. coli O157:H7 in cookie dough. Samples of artificially inoculated cookie

dough were incubated at 37°C for up to 24 hours. In culture method, Salmonella and E. coli O157:H7 were detected by selective plating on selective media. In multiplex PCR, invA and hilA genes were used to detect Salmonella; stx1, stx2, and rfbE gene were used to detect E. coli O157:H7. For real-time PCR, SureTect Pathogen Detection kit was used with PikoReal real-time PCR system for both pathogens. While various concentrations of pathogens in cookie dough were tested, we also compared the total assay time required to detect 1 CFU/20 g for each assay. The results indicated that culture methods were able to detect 104 CFU/20g Salmonella and 103 CFU/20g E. coli O157:H7 in 24 hours. Multiplex and real-time PCR identified both pathogens at 105 CFU/20g after 8 hours and 1 hour, respectively. In order to detect 1 CFU/20g of pathogens in cookie dough, culture method required 33 hours and 36 hours for Salmonella and E. coli O157: H7, respectively. Multiplex PCR were able to detect 1 CFU/20 g of pathogens in 14 to 20 hours including enrichment. For real-time PCR, 1 CFU/20g of both pathogens were detected in 14 hours. This study demonstrated that culture method and both PCR methods were reliable to detect Salmonella and E. coli O157:H7 in raw cookie dough. However, PCR-based methods were able to detect pathogens in cookie dough much more rapidly than culture-based method. PCRbased methods would be a good alternative to standard culturebased method for pathogen identification in cookie dough products for efficient product monitoring.

03. BUILDING A HIGH RESOLUTION SPATIAL DATASET TO ASSESS THE HETEROGENEITY OF DIARRHEAL PREVALENCE AND RISK IN EAST AFRICA

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Diarrheal diseases are a major cause of childhood (<5 years) mortality worldwide, with nearly half of deaths occurring in Africa. Although global mortality from diarrhea is declining, much of Africa experiences a disproportionate burden of these treatable diseases. Unequal access to adequate nourishment and healthcare among socioeconomic groups contributes to disease burden. Undernourished children are highly susceptible and experience higher morbidity and mortality from diarrhea. Climate change is projected to increase climate variability, and frequency of extreme events (e.g. floods and droughts). As agriculture in much of sub-Saharan Africa is heavily reliant on rainfall and weather patterns, precipitation alterations may influence agricultural production and availability of adequate nourishment for children. Previous studies have shown relationships between climate and diarrheal prevalence, undernutrition, or demographic changes, but have largely focused on illustrating large-scale patterns across entire countries or regions of Africa. Here, we explore the heterogeneity of diarrhea occurrence and risk factors in East Africa at a finer spatial resolution. We also are constructing a dataset to build spatial regression and predictive models to explore the relationship between diarrheal prevalence in children < 5 in East Africa using social, environmental, and anthropomorphic variables.

04. CHITOSAN MICROPARTICLES KILL ANTIBIOTIC RESISTANT MICROORGANISMS WITHOUT RAISING DETECTABLE RESISTANCE

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Antibiotic resistance is growing exponentially, increasing public health concerns for humans and animals. In the current study, we investigated the antimicrobial features of Chitosan microparticles (CM), engineered from chitosan by ion gelation, seeking potential application for treating infectious disease caused by multi-drug resistant microorganisms. CM showed excellent antimicrobial activity against a wide range of microorganisms, including clinically important antibiotic resistant bacterial pathogens without raising resistant mutants against CM in serial passage assays over a period of 15 days, which is a significantly long passage compared to tested antibiotics used in human and veterinary medicine. In addition, CM treatment did not cause cross-resistance, frequently observed with other antibiotics, triggering occurrence of multi-drug resistance. Antimicrobial activity of CM was exceptionally strong to eliminate pathogens completely. Furthermore, CM activity was examined in simulated gastrointestinal fluids to mimic the environment that CM would encounter when orally administered. CM at a concentration of 0.00001% killed E. coli O157:H7 completely in synthetic gastric fluid within 20 minutes. Risk assessment of CM, in an in vitro animal

model, revealed that CM did not disrupt the digestibility, pH or total volatile fatty acid production, indicating that CM may not affect the functionality of the rumen. Given all the above listed advantages, CM can serve as a great candidate to treat infectious disease, especially those caused by antibiotic resistant pathogens without adverse side effects.

05. CHOLERA IN CAMEROON, 2000-12: SPATIAL AND TEMPORAL ANALYSIS AT THE OPERATIONAL (HEALTH DISTRICT) AND SUB CLIMATIC LEVELS

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Background: Recurrent cholera outbreaks have been reported in Cameroon since 1971. However, case fatality rates remain high, and we do not have an optimal understanding of the epidemiology of the disease, due in part to the diversity of Cameroon's ecologic regions and the lack of a comprehensive country-wide analysis at the district level.

Methods/Findings: We undertook an analysis of the epidemiology of cholera in Cameroon by using a unique district-level dataset of cholera case numbers and deaths from 2000-2012, obtained from the Ministry of Public Health and World Health Organization country office. During this time period, 43,474 cholera cases were reported: 1748 were fatal (annual mean case fatality rate7.9%), with an attack rate of 17.78 per 100,000 inhabitants per year. Outbreaks occurred in three waves during the 12 year time period, with case fatality rates peaking at the beginning of each wave. There was a clear separation in time and space between cases in the northern and southern portions of the country. When sub-divided by ecologic zone (Sudano-Sahelian, tropical humid, Guinea equatorial, and equatorial monsoon) there were strikingly different seasonal patterns of illness: in the Sudano-Sahelian ecologic zone in the northern portion of the country cases tended to occur between July and September, during the rainy season, while in the equatorial monsoon region in the south, cases were seen year-around, with the lowest case numbers in the July-September period, when rains were peaking. In a spatiotemporal cluster analysis, multiple case clusters could be identified. Occurrence of cases was significantly linked with the presence of coastline, inland water areas, and major highways.

Conclusions/Significance: The epidemiology of cholera in Cameroon varies dramatically based on ecologic zone. Development of global control strategies for cholera will not be possible without an understanding of the impact of local ecology and transmission patterns on disease occurrence, particularly in the setting of ongoing climate change.

06. COMPARATIVE GENOMIC ANALYSIS OF BACTERIA HARBORING EXTENDED SPECTRUM B-LACTAMASE GENES ISOLATED FROM CATTLE AND ENVIRONMENTAL SOURCES ON FARMS

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According to the Centers for Disease Control and Prevention, there are at least 2,049,224 illnesses and 23,000 deaths attributed to antibiotic resistance each year. In 2003, the Food and Agriculture Organization and World Health Organization released a statement indicating that the major transmission pathway of resistant bacteria and their genes from the agricultural environment to humans is through consumption of food-producing animals. The purpose of this study was to compare antibiotic resistance genes, specifically extended-spectrum β-lactamase (ESBL) genes, among bacteria isolated from commercial beef farms in North Florida. Fecal samples were collected from two separate cohorts of multi-breed beef calf populations derived of Brahman and Angus cattle in 2013 and 2014. Neither set of calves had been previously exposed to antibiotics. Fecal samples were serially diluted and plated on MacConkey Agar containing 4 µg/ml cefotaxime to isolate ESBL-producing bacteria. Whole genome sequencing (WGS) of 31 isolated cefotaxime resistant bacterial isolates from cattle was performed using the Illumina MiSeq. De novo genome assembly was performed using Galaxy and whole genomes were annotated using RAST. WGS revealed that the major bacterial species represented by resistant bacteria isolated

from farms was Escherichia coli. The majority of isolates from 2013 were found to contain the blaCTX-M-1 gene while 2014 isolates were found contain blaCTX-M-32 genes. In addition, all isolates from 2014 were found to be multi-drug resistant compared to 11.8% of isolates from 2013. The results of this study demonstrate the high levels of antibiotic resistant organisms shed by cattle not previously exposed to antibiotics. This study also shows the evolution of antibiotic resistance in bacterial populations in food-producing animals over time.

07. COMPARATIVE WHOLE GENOME SEQUENCE ANALYSIS OF LONG-TERM SURVIVING VIBRIO CHOLERAE IN NUTRIENT-POOR LAKE WATER MICROCOSMS.

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Vibrio cholerae, the causative agent of the water-borne disease cholera, can shift to Growth-advantage stationary phage (GASP) phenotype in a stationary growth condition. We have previously reported a "GASP" phenotype that survived in nutrient-poor "filter sterilized" lake water (FSLW) microcosms for up to 700-days.

Furthermore, we observed that the GASP persisting cells in 700-day old microcosms produced biofilms unique to FSLW. Identifying the sustained mutations in Vibrio cholerae GASP phenotype will enhance our understanding of how this phenotype is selected under stressful conditions that promote its environmental fitness and adaption. Here we report whole genome comparative analysis of wild-type V. cholerae El tor strain N16961, together with the same strain from a 24-hour microcosm (N16961-24), 190 day-old microcosm and a 700day old microcosm. Single nucleotide polymorphisms (SNPs) and INDELs were identified by using both reference mapping and de novo assembly approaches. A bioinformatic pipeline was optimized for cholera genomic analysis in Galaxy. In particular, Freebayes was used to detect the variants and the SnpEff tool for annotating the variants and predicting their effect, while de novo assembly of the sequence reads was obtained with multiple alignments of whole genomes using MAUVE. Our analysis shows that compared to the wild type V.cholerae N16961 El tor strain, all three GASP phenotypes exhibited a total of 191 high quality mutations, with 50 mutations identified in the coding regions in both N16961-24 and 190 day-old strains and 51 mutations in the 700-day old GASP genome; out of which 28 (55%), 24 (48%) and 28 (52%) were due to INDELs respectively, causing frameshift in several open reading frames. Point mutations consisted of about 20 non-synonymous and 3 synonymous changes in all three GASP phenotypes. Among the genes exhibiting these changes, important regulatory proteins are included such as the CsrD, Ribosome GTPase, GGDEF and (p) ppGpp synthetase. In conclusion, our comparative analysis sheds light on significant genomic changes occurring in the long-time surviving GASP phenotypes, some of which may be responsible for the adaptation of the bacteria to stressful and stationary growth conditions.

08. DISTRIBUTION OF VIABLE AND CULTURABLE VIBRIO CHOLERAE IN MAGA LAKE, FAR NORTH REGION OF CAMEROON: PRELIMINARY FINDINGS

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Cholera has been endemic in Cameroon since 1971 and the northern part of the country (e.g. the North and Far North regions) is considered one of the cholera epicenters. The 2010-11 major cholera outbreak hit all over Cameroon and it was speculated that the outbreak was initiated in the Far North Region and spread throughout the country. However, little is known on how the outbreak took place and how the disease spread. In this study we aim to explore potential environmental reservoir of Vibrio cholerae in the Far North Region with a focus on Maga Lake. Maga Lake is located in a large plain of the Far North Region and floods each year. The people living around the Maga Lake are among the most affected population by cholera during the 2010-2011 outbreaks. In July 2011during the rainy season, we selected three sites in Maga Lake and collected samples thrice from various sources (500 mL of water, 50 g of sediments, fish) at different sampling points and the samples were examined for contamination with V. cholerae. Other microbiological parameters and physicochemical characteristics (pH, temperature, salinity and turbidity) of the water were also measured. The Spearman's Rank correlation was used to analyze the relationship between physico-chemical factors and the occurrence of V. cholerae. Results showed that the abundance of heterotrophic aerobic and mesophilic bacteria (1.60±0.7x106 UFC/mL) and bacterial bio-indicators in lake water all exceeded the WHO's recreational water standards. Faecal streptococci were present in all water samples at high concentrations (31±0.64 UFC/100 mL). In sediments, the abundance of heterotrophic aerobic and mesophilic bacteria reached 2.07±0.06x1010 UFC/mL. Vibrio metschnikovii was the only species of Vibrio genus found in water samples. However, sediments were contaminated with three species of Vibrio genus: V. parahaemoliticus, V. metschnikovii and V. cholerae. We found V. vulnificus and V. alginolyticus from fish samples. Further investigations are needed to understand how viable and culturable V. cholerae moves from one compartment of the lake to another and how flooding modulates cholera transmission from the Maga Lake.

09. ENVIRONMENTAL FACTORS OF ENDEMISM OF CHOLERA IN THE NORTH CAMEROON: PRELIMINARY RESULTS

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Cholera has been endemic in Cameroon since 1971 with an overall increasing trend. In the Northern Cameroon, cholera outbreaks regularly occur every year during the rainy season. Nevertheless, how the outbreak takes place and how the disease is transmitted in the region remain largely unknown. In this study, we aim to characterize water quality of major drinking water sources in 2

regions (North and Far North) of Cameroon and explore potential environmental reservoir of the causative agent, Vibrio cholerae, and factors maintaining its persistence. Here we report some preliminary findings.

69 water sources from 17 quarters in North (four in Bibémi, six in Mayo Oulo subdivisions) and Far North (one in Maroua 1, four in Maroua 2, two in Mokolo subdivisions) of Cameroon were chosen according to their vulnerability to cholera outbreaks. Water samples were collected from wells and streams and analysed twice during the rainy season from June 2015—November 2015. Those samples were analysed for Vibrio cholerae contamination and to enumerate qualitative microbial indicators (faecal coliforms) according to the standard methods. The physico-chemical parameters considered were pH, electrical conductivity, temperature, total dissolved solids (TDSs) and salinity. These parameters were chosen in accordance with their general importance in bacterial metabolism and the availability of our laboratory equipment.

The results show that faecal coliforms were present in all well and stream water samples at high concentrations (3.3x104 to 7.3x104 CFU/100 mL and 3.1x104 to 1.2x105CFU/100 mL of water, respectively). Physico-chemical measurements showed that water temperature varied from 25.7 to 31.8°C. Water pH varied from 6.98 to 7.89 with low and high mineralization. Of the water sources investigated, 3 (2 wells and 1 stream) were positive for V. cholerae and 18 isolates were collected. Strains and pathogenic potential of the isolates were not yet determined. The positive water samples were from Doualaré (Maroua 2), Hardé (Maroua 1) and Bibémi. 75 households per subdivision were surveyed for information on water management and sanitation. The discharge to the environment of sewage drains by 24.07 % of households, the absence of latrines in 8.33 % of households and the use of materials placed on the soil to collect water by 89.81 % of households render these water resources

vulnerable. These factors, coupled with rainfall, might play critical roles in the distribution of faecal matters and water contamination.

10. EVALUATION OF RAPID DETECTION METHODS OF SALMONELLA ENTERITIDIS AND E. COLI 0157:H7 IN COOKIE DOUGH

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Cookie dough is recognized as a potential vehicle for Salmonella, and was associated with E. coli O157: H7 outbreaks in 2009 as a novel vehicle. It is believed that the nature of cookie dough of high fat and low water activity can provide protection pathogens' survival during storage. It is important to study what role each ingredient plays in such effect. This study aims to determine the survival of foodborne pathogens in cookie dough during storage, and to assess the impact of each main ingredient and different fats used in cookie dough on survival of pathogens. For this goal, commercial cookie dough was artificially inoculated with S. Enteritidis and E. coli O157:H7 (106 CFU/g). Samples were stored at 4°C and -18°C and the number of surviving cells were counted for 8-week period. Cookie dough samples were also prepared with various fat, sugar and salt contents, and with four different fats including margarine, butter, coconut oil, and olive oil. These samples were inoculated with S. Enteritidis and

E. coli O157:H7 (106 CFU/g) separately, and the survival of pathogens was determined for 8 weeks. After 8 weeks, 2.42 and 2.35 log reduction for Salmonella and 2.23 and 1.99 log reduction for E. coli O157:H7 were obtained at 4°C and -18°C, respectively. Cookie dough prepared with seven different recipes of cookie resulted in reduction of both pathogens, ranging from 0.73 to 1.95 log CFU/g. Overall, both pathogens had lower survival rate in samples in higher sugar content. Significant reduction of Salmonella cell count was observed in samples with higher fat. Besides, overall Salmonella concentration was lower in sample containing coconut oil, and E. coli O157:H7 was lower in samples containing olive oil.

Our study suggests that each ingredient has different impacts on the pathogen survival. Changing some of ingredients could reduce the survival of S. Enteritidis and E. coli O157:H7. However, changing storage conditions and ingredients failed to completely eliminate pathogens. Good manufacturing practices and consumer education are essential to prevent future outbreaks.

11. GENETIC STUDIES OF VIBRIO CHOLERAE IN SOUTH WEST CAMEROON—A PHYLODYNAMIC ANALYSIS OF ISOLATES FROM THE 2010-2011 EPIDEMIC

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During the cholera outbreak from 2010 to 2011 in Cameroon, 33,192 cases with 1,440 deaths (case fatality rate 4.34%) were reported to the World Health Organization (WHO). Of these, the South West Region (SWR) reported 3,120 clinical cases. This region is in the Equatorial Monsoon climatic region of Cameroon, close to the coast, raising questions as to whether cases were linked with development of environmental reservoirs. In an investigation conducted by the Laboratory for Emerging Infectious Diseases, University of Buea, toxigenic V. cholerae O1 were isolated from diarrheal stool samples from 18 patients, with ages ranging from <3 to 70 years. Coordinates for clinical centers at which cases were identified were obtained using a handheld GPS, and were mapped using ArcGIS; locations are shown in the left panel of the Figure, below.

The full genomes of these strains were sequenced with the Illumina MiSeq platform. De novo assembly of cholera genomes and multiple sequence alignment were carried out using the bioinformatic pipeline developed in the EPI laboratory at University of Florida [Azarian et al., Mbio 2014]. Genetic comparisons showed that isolates were closely related, with pairwise p-distances ranging from 2.25 to 14.52 10-5 nt substitutions per site, and no statistically significant correlation between the pairwise genetic distances and the geographic distances among sampling locations. Indeed, the phylogeny of the Cameroonian strains displays the typical star-like topology and intermixing of strains from different locations that are characteristic of an exponential outbreak localized around a relatively restricted area with occasional spillover to other parts of the country, likely mediated by direct human contact and human

movement. In contrast, movement of strains through local environmental reservoirs might be expected to show a correlation between genetic and geographic distance. Findings highlight the utility of whole genome sequencing and phylodynamic/phylogeogrpahic analysis in understanding transmission patterns at the local level.

12. HEAT RESISTANCE OF SPORES OF SIX MAJOR BACILLUS SPECIES IN PHOSPHATE BUFFER AND FERMENTED SOYBEAN PRODUCTS

<u>Jae-Hyung Mah</u> - Korea University; **Xuezhi Bai** - Korea University; **Young Kyoung Park** - Korea University

The aim of this study was to compare the heat resistance of Bacillus cereus spores with that of spores of dominant Bacillus species present in fermented soybean products and thereby optimize thermal treatment conditions of fermented soybean products to reduce risk of B. cereus contamination. For this, heat resistance of spores of six different species belonging to the genus Bacillus, including B. subtilis, B. coagulans, B. licheniformis, B. pumilus and B. brevis as well as B. cereus, was measured in a phosphate buffer. Consequently, B. cereus spores revealed the smallest D-values at all the tested temperatures of 100-112°C, whereas B. licheniformis spores had the greatest D-values at temperatures in the range of 106-112°C. The spores of the other dominant Bacillus species showed similar or slightly smaller D-values than those of B. licheniformis at respective temperatures. Subsequently, heat resistance of spores of six species was determined in two types of fermented soybean products, Cheonggukjang and Gochujang products. In the fermented soybean products, B. licheniformis spores revealed the greatest D-value at 112°C, and one of either B. coagulans spores or B. brevis spores exhibited the greatest D-values at temperatures in the range of 103-109°C, whereas B. cereus spores showed the smallest D-values at all the tested temperatures.

Meanwhile, B. licheniformis spores had the highest z-values in both phosphate buffer and fermented soybean products, followed by B. cereus spores. These results indicate that a mild thermal treatment would allow species-selective inactivation of B. cereus spores, less affecting spores of the other Bacillus species.

13. IMPLEMENTATION OF A NEW ALGORITHM FOR THE IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI (STEC)

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Background: The Centers for Disease Control and Prevention (CDC) estimates that STEC bacteria are responsible for 37,000 illnesses, 1,100 hospitalizations and 30 deaths annually in the U.S. Over the past year, the Florida Department of Health, Bureau of Public Health Laboratories (BPHL) has modified their STEC algorithm to decrease screening cost for detection of STEC while improving capacity and also adding the ability to characterize and isolate non-O157 STEC organisms.

Methods: BPHL utilized the Enzyme Immunoassay (EIA) method for the detection of Shiga-toxin in broth, with isolation and characterization of only O157:H7 serotype. Modifications include replacing EIA with a four-plex PCR for the detection of stx1, stx2, eaeA, and ehxA (hlyA), and adding CHROMagar STEC media for the isolation of O157:H7 STEC and non-O157 STEC organisms. Antisera to

serotypes O26, O45, O103, O111, O121, O145, are then used to further isolate/characterize these six non-O157 serotypes.

Results: The replacement of EIA with a PCR method has helped reduce costs from \$10 to \$4 per/test. The addition of CHROMagar STEC and the individual "Big Six" antisera has improved our ability to detect more serotypes than just O157. In the past year BPHL has identified a significant number, n=49, of STEC from non-O157 serogroups and has done so in a timely manner with an average turnaround time of 5-10 days.

Conclusion: The isolation of STEC from patient stool in broth is a challenge. This challenge will increase with the increasing number of culture-independent tests being performed. BPHL's new algorithm, utilizing a multiplex PCR instead of EIA, has increased our STEC detection rates and reduced our cost per/test. The increase of positivity in broths led to the CHROMagar STEC and "Big Six" antisera additions which not only increase our isolation of the causative organism but help us characterize the most common non-O157 STEC organisms reducing the time it takes to report the final identification.

14. MICROBIAL QUALITY OF SURFACE AGRICULTURAL WATER AND THE PRESENCE OF PATHOGEN GENES IN CENTRAL FLORIDA

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How the microbiological quality of surface water relates to, and should be evaluated, in regard to produce safety requires further evaluation.

The purpose of this study is to determine populations of indicator organisms and the presence of Salmonella and Shiga Toxin producing Escherichia coli (STEC) genes in agricultural water.

Water samples (500 ml) from six agricultural ponds were collected during the 2012/2013 and 2013/2014 growing seasons (46 and 44 samples respectively, 540 total). Microbial indicator populations (total coliforms, generic Escherichia coli, and Enterococci) were enumerated. A microbial water quality profile (WQP) was established for all ponds. Water (150 ml) was filtered and filters stored at -20°C until pathogen analysis by PCR. For STEC, filters were enriched in modified buffered peptone water with pyruvate at 35±2°C for 24 h, DNA extracted, and multiplex PCR for detection of six genes (hly, fliC, eaeA, rfbE, stx-I, and stx-II), run. For Salmonella, the presence of the invA gene was evaluated following a subsequent enrichment in Rappaport-Vassiliadis 42±1°C for 48 h and DNA extraction.

All ponds met the current FDA WQP recommendations 100.0% of the time. All STEC genes were detected in 2.6% of the samples. Individual STEC genes varied in the number of samples they were

detected in: hly-83.3%, fliC-51.8%, eaeA-17.4%, rfbE-17.4%, stx-I-32.6% stx-II-9.4%. The invA gene was detected in 26/540 (4.8 %) samples, in all ponds and both growing seasons. However, 57.7 % (15/26) of the invA positive samples were from ponds 2 and 4, where the WQP was the poorest.

Surface waters tested in Central Florida meet the FDA recommendations for microbial water quality, however at least one Salmonella or STEC gene was detected in 91.3% of samples. Understanding the relationships between indicator microorganisms and pathogens presence allows a greater understanding of agricultural water risks.

15. OPTIMIZATION AND STRAIN VARIATION FOR THE REDUCTION OF SALMONELLA ENTERICA BY CHITOSAN MICROPARTICLES

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Introduction: Contamination of agricultural waters by Salmonella enterica presents a challenge to the food industry. Chitosan microparticles (CM) have shown broad-spectrum antibacterial activity against numerous species, including S. enterica; however, various environment parameters may alter the potential for its application to food protection.

Purpose: In this study anti-Salmonella activity of CM was determined for different CM concentrations and various environmental

conditions in order to optimize its efficacy. Strain variation in CM sensitivity was examined, and genomic comparisons investigated genes and genotypes and their relationship to CM sensitivity.

Methods: Growth and survival of a three-strain S. enterica cocktail was determined by mean log CFU/ml ± standard deviation in water, pond water, artificial seawater (20 ppt), and nutrient broth with or without addition of various concentrations of CM at different pH (5,7,9) and temperature (25, 30, 37°C) combinations. Survival of various S. enterica strains and serotypes was compared under optimized conditions, and genomic sequences of these strains were processed by Bowtie, and Rapid Annotation Subsystem Technology in order to determine phylogenies and annotation of contig files.

Results: Optimum conditions for CM activity against S. enterica in water were pH7 and 37°C, but the effects of CM were significantly (P<0.0001) diminished by addition of NaCl, the presence of complex bacterial communities in pond water, and in stationary compared to log phase growth. S. enterica strains were nondetectable after 2 h exposure to 0.3% CM in sterile water, and significant reductions were seen with CM concentrations as low as 0.01% (p<0.001). Strains differences in CM sensitivity were observed and varied within serotype, while corresponding genotypes and predicted proteins were associated with increased sensitivity to CM.

Significance: This study provides conditions that may be useful for the application of CM as a sanitizer for irrigation and agricultural wash water.

16. OPTIMIZATION OF FABRICATION FOR CHITOSAN-DRIVEN NANOPARTICLES TO ENHANCE ANTIMICROBIAL ACTIVITY AGAINST FOODBORNE PATHOGEN ESCHERICHIA COLI 0157:H7

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Chitosan nanoparticles (CN) have been developed as a natural antimicrobial agent with broad-spectrum antimicrobial activity. Many different types of CN have been generated using a variety of chitosan sources, cross-linkers, and sonication conditions. However, not many previous studies have comparatively assessed the antimicrobial activity of different types of chitosan nanoparticles against foodborne pathogens. The purpose of this study was to evaluate different engineering methods for production of CN to enhance its antimicrobial activity, which will help optimize the potential for further application of these particles. CN was prepared in solution using different molecular weights of chitosan, cross linkers (sodium sulfate or tripolyphosphate) and sonication conditions. The size of CN was measured using a nanoparticle analyzer. The antimicrobial activity of CN was assessed against E. coli

O157:H7 to determine the optimal conditions for chitosan nanoparticle generation with high antimicrobial properties. It was observed that CN with a size smaller than 150 nm exerted better antimicrobial activity. In addition, the selection of sodium sulfate as a crosslinker over sodium tripolyphosphate achieved better results, as most bacteria were killed after 4 h and no regrowth was observed after 24 h. For sonication power, 60 W enhanced the antimicrobial activity compared with 96 W. The best engineering conditions that enhanced antimicrobial activity included a combination of low molecular weight chitosan with sodium sulfate as cross-linking agent at a final concentration of 0.4-0.6 %. The optimized engineering of CN particles may be applied in future studies to assess their applicability in different fields, such as animal disease treatment as well as elimination of other pathogens from food and the environment.

17. PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA AND E. COLI O157:H7 FROM ANIMAL AND ENVIRONMENTAL SOURCES IN NORTH FLORIDA

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According to the Food and Agriculture and World Health Organizations, the primary route of transmission of antibiotic resistant organisms from the environment to humans is through food. It is generally accepted in the scientific community that the emergence of antibiotic resistant bacteria is due to the use of agricultural growth promotors in animal feed however; levels of antibiotic resistant bacteria continue to rise in food-producing animals not previously exposed to antibiotics. The purpose of this study was to determine the prevalence of extended spectrum β lactamase-producing (ESBL) antibiotic resistant bacteria and E. coli O157:H7 in animal and environmental sources on commercial cowcalf operations in North Florida. Over 1000 animal and environmental samples were collected from 17 commercial beef farms across North Florida. ESBL-producing bacteria were enumerated from samples by plating onto MacConkey Agar supplemented with 4 µg/ml of cefotaxime. E. coli O157:H7 was enumerated from samples by plating onto sorbitol MacConkey agar supplemented with cefixime and tellurite. The average prevalence of ESBL-producing bacteria and E. coli O157:H7 on farms was 4.3 and 4.0 log CFU/g, respectively. Levels of ESBL producing bacteria were significantly higher in soil samples (P=0.002) and forage samples (P=0.03). Prevalence of E. coli O157:H7 was highest in forage samples with a concentration of 5.6 CFU/g (P=0.01). For both ESBLproducing bacteria and E. coli O157:H7, prevalence was lowest in water samples with a concentration of 1.2 CFU/ml and 0.5 CFU/ml,

respectively. These results suggest that transmission of antibiotic resistant organisms to food-producing animals may occur through environmental sources, specifically soil and forage. In addition, emergence of antibiotic resistant bacteria may occur naturally in the environment.

18. PROTEOMIC ANALYSIS OF EXTRACELLULAR PROTEINS OF THP-1 MACROPHAGES INFECTED WITH SALMONELLA ENTERICA TYPHIMURIUM

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The host–bacteria interactions involve complex alteration of protein networks, which are controlled by both the host and the pathogen. Secreted and other extracellular proteins can act as important messengers in the intercellular communication and they can stimulate the immune response to the pathogens. Salmonella enterica Typhimurium is known to induce a strong inflammatory response in the host cell. This bacterial infection leads to an increase in secretion of various chemokines and growth factors, and it interferes with the host secretory pathways. In this study, we describe the extracellular proteome of human macrophages infected with Salmonella Typhimurium, followed by analysis of molecular functions and canonical pathways of proteins isolated from the extracellular milieu, which abundance was affected by infection. Finally, we provide an evidence of the extracellular vesicles produced by human macrophages, which transport specific protein cargo from infected cells at early stages of infection.

19. RISK ASSESSMENT OF INTESTINAL EPITHELIAL CELLS AND CAENORHABDITIS ELEGANS EXPOSED TO CHITOSAN NANOPARTICLES

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A natural antimicrobial agent, chitosan nanoparticles (CN), has previously demonstrated broad-spectrum antimicrobial activity. CN have many potential applications in the food industry and offers an alternative to traditional antibiotics. However, it has not been determined whether CN may cause adverse side effects in humans and animals. The purpose of this study was to evaluate the toxicity of CN toward intestinal epithelial cells, Caco-2 cells, and an animal model, C. elegans. Four types of CN were prepared by cross-linking of chitosan. Two crosslinkers, sodium sulfate (SS) and tripolyphosphate (TPP), and two types of chitosan, low and high molecular weight, were used to generate CN. Caco-2 cells were treated with 0.1, 0.2 or 0.4% CN for 24 h. The morphological change of the cells was checked immediately following treatment. Cellular membrane damage was assessed by lactate dehydrogenase (LDH) assay. In addition, the four types of CN from 0.1 to 0.4% were administered to C. elegans for survival assay. The viability of C.

elegans was monitored every two days for 22 d. No change in cell morphology or cell viability was observed in Caco-2 cells treated with CN compared to the control. However, we observed a mild level of toxicity in the animal model. In the C. elegans survival assay, CN generated with TPP showed lower toxicity compared to the ones generated with SS. The molecular weight of chitosan did not affect the toxicity. This data demonstrates that CN are not toxic toward intestinal epithelial cells, but are toxic in the animal model. These results will help with future development of animal models for the risk assessment of newly developed agents.

20. SOCIO-ECONOMIC, ENVIRONMENTAL AND WATER, SANITATION AND HYGIENE DISPARITIES IN PERI-URBAN KENYA.

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Background: Diarrhea is a leading cause of child death, internationally and in Kenya. The impact is primarily seen in the poor and vulnerable. Coupled with socio-economic factors and other disparities, the risk factors for diarrheal disease mortality include poor water and sanitation (WASH), nutritional fragilities and inadequate treatment.

Aims: 1. To describe the social, economic and environmental factors that contribute to WASH related behavior and conditions at both the community and household level.

- 2. To estimate and describe the relationship between conditions and behaviors at the household and community level, and contamination of key exposure points.
- 3. To describe exposure pathways from measured household and community variables to detect pathogens in children's feces via measured contamination of exposure points.

Methods: This study is based in 3 peri-urban settlements in Kisumu. These include: Nyalenda A, B and Kanyakwar, bordering the shores of lake Victoria in western Kenya. The study was done in 2 phases.

Phase 1: Qualitative data was collected using purposive random sampling methods, by which 14 focus group discussions with mothers, landlords and tenants, 20 semi-structured interviews with mothers of children aged 6-36 months as well as 6 transect walks were conducted with general communities members to understand the various demographic, socio-economic and environmental determinants of WASH conditions and its outcomes on the communities.

Phase 2: A household survey of 800 households was conducted using a two-stage probability cluster sampling design. Data was collected on demographics, socio-economics, household WASH behaviors, recent diarrhea and housing structures. Child and animal stool, drinking water, child food, kitchen surface swabs, and fly samples were also collected from each compound of the sampled households. Additionally, height and weight measurements for all children aged 6 – 36 months were recorded and GPS location points were collected for each of the households studied.

Conclusion: This is a multi-partner study with University of Florida, London School of Hygiene and Tropical Medicine, Great Lakes University Kisumu and Kenya Medical Research Institute

collaborating to provide beneficial opportunities for scientific scholarship. These data are currently being analyzed.

21. THE SPATIO-TEMPORAL RELATIONSHIP OF DYSENTERY CAUSED BY SHIGELLA

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Background: The geographic and spatial distribution of the Shigella bacteria varies greatly throughout the world by species and serotype. The Center for Disease Control and Prevention reports that in the United States alone, there are an estimated 500,000 cases of Shigellosis every year with an average annual incidence of 4.82 cases per 100,000 individuals in 2013. This review aims to investigate the spatio-temporal relationship of dysentery caused by Shigella.

Methods: A thorough search of the literature was performed in order to identify the articles chosen for this review. The search was performed in Pubmed, Web of Science and specific journals including: The International Journal of Health Geographics and The Journal of Spatial and Spatio-Temporal Epidemiology. A search on EbscoHost and a general UF library search were also performed. The search aimed at identifying the different types of spatio-temporal risk factors for dysentery. These risk factors were later summarized and added to a representation of the epidemiologic triangle.

Results: We were able to identify different spatio-temporal risk factors for dysentery caused by Shigellosis. Several articles included elements from the three parts of the epidemiologic triangle, demonstrating the interrelationship of the environmental and human factors during outbreaks of Shigellosis.

Conclusions: In line with previous research, our study found that the dysentery susceptible population includes a variety of people and it is present throughout the world both in developed and developing countries.

22. UTILIZATION OF PACBIO WHOLE GENOME SEQUENCING TO CHARACTERIZE A HYPER-VIRULENT ESCHERICHIA COLI O157:H7 STRAIN ISOLATED FROM SUPER-SHEDDING CATTLE

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Shiga toxin-producing Escherichia coli (STEC) O157:H7 is a foodborne pathogen that threatens public health on a global scale. STEC O157 predominantly colonizes the terminal recto-anal junction (RAJ) of cattle, which is the major asymptomatic reservoir of this pathogen. Cattle shedding O157 ≥ 104 CFU/g of feces are known as supershedders and are responsible for within-farm and between-farm transmission of STEC O157. The purpose of this study was to perform genetic characterization of KCJ1266, a strain isolated from a supershedder steer from a farm in North Florida. PacBio sequencing was employed for whole genome sequencing (WGS) to characterize the genomic features of KCJ1266. A comparative genome analysis of

KCJ1266 with reference genomes including SS17 (strain isolated from a super-shedder), EC4115 (strain related to spinach outbreak) and EDL933 (strain related to hamburger outbreak) was conducted. WGS of KCJ1266 revealed that it has a genome of 5,478,683 bp encoding 5,545 open reading frames and a plasmid, pO157, of 95,910 bp. In silico analysis revealed that KCJ1266 belongs to E. coli Lineage I/II and clade 8, which are related to other disease-causing isolates. In addition, Mauve alignment showed that KCJ1266 shares a similar genomic architecture with SS17 and EC4115. Comparative analyses also revealed that KCJ1266 has the same virulence and similar functional genes as SS17 and EC4115. Phylogenetic analysis showed that KCJ1266, SS17 and EC4115 clustered in the same group. Taken together, these results reveal that super-shedding STEC strain KCJ1266 is a hyper-virulent strain similar to that of SS17 and EC4115. This is one of the first studies which utilizes PacBio WGS to characterize a hyper-virulent STEC O157 strain isolated from supershedding cattle. KCJ1266 can potentially be used as a reference strain for future studies regarding the phenomenon of supershedding.

23. WHOLE GENOME ANALYSIS OF ESCHERICHIA COLI O157:H7 REVEALS UNIQUE GENETIC FEATURES THAT MAY ENABLE BACTERIA TO COLONIZE PROFICIENTLY IN HOSTS

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Shiga toxin-producing Escherichia coli (STEC) O157:H7 is an important foodborne pathogen causing outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (HUS). Cattle are a major asymptomatic reservoir of STEC O157 and this pathogen colonizes primarily in the terminal recto-anal junction (RAJ). Cattle shedding O157 of more than 104 CFU/g of feces are regarded as supershedders. Since STEC O157 is able to survive in animals, drinking water, and feces, it can be transmitted easily to other hosts. Predominant STEC 0157 strains, which are well adapted to hosts and environments, are responsible for a large part of O157 outbreaks. Previous studies have revealed that an E. coli O157:H7 subtype strain (FRIK2455) was predominant on a farm 2 while other clonal variants were rarely isolated (FRIK2133 and FRIK2533). However; genetic factors of these predominant isolates that may explain their dominance in cattle are not understood. In this study, we conducted whole genome sequencing to identify genetic factors that may confer predominance in cattle. By conducting comparative genome analysis of those genomes, we found that these strains share similar genetic composition and structure, but distinct features in plasmids. Only the predominant strain FRIK2455 carries a plasmid, p35K, that encodes a type IV secretion system (T4SS) that may provide an advantage for survival in hosts and environments. Further analysis

will focus on understanding molecular mechanisms of T4SS in the predominant strains in hosts.

24. DISPARITIES IN INFLUENZA MORTALITY AND TRANSMISSION RELATED TO SOCIO-DEMOGRAPHIC FACTORS WITHIN CHICAGO IN THE PANDEMIC OF 1918

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Social factors have been shown to create differential burden of influenza across different geographic areas. We explored the relationship between potential aggregate-level social determinants and mortality during the 1918 influenza pandemic in Chicago using an historical dataset of 7971 influenza and pneumonia deaths. Census tract-level social factors, including rates of illiteracy, homeownership, population, and unemployment, were assessed as predictors of pandemic mortality in Chicago. Poisson models fit with Generalized Estimating Equations (GEE) were used to estimate the association between social factors and the risk of influenza and pneumonia mortality. The Poisson model showed that influenza and pneumonia mortality increased on average by 32.2% for every 10% increase in illiteracy rate, adjusted for population density, homeownership, unemployment, and age. We also found a significant association between transmissibility and population density, illiteracy, and unemployment, but not home-ownership. Lastly, analysis of the point locations of reported influenza and pneumonia deaths revealed fine-scale spatiotemporal clustering. This study demonstrates that living in census tracts with higher illiteracy rates

increased the risk of influenza and pneumonia mortality during the 1918 influenza pandemic in Chicago. Our observation that disparities in structural determinants of neighborhood-level health lead to disparities in influenza incidence in this pandemic suggests that disparities and their determinants should remain targets of research and control in future pandemics.

25. INFLUENZA INFECTIVITY MODULATION BY CARBON NANOPARTICLES ON SIALIC ACID

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Nanoparticles have unique characteristics that provide value to a growing number of consumer industries. Single-walled carbon nanotubes (SWNTs) are cylindrical allotropes of carbon that have previously been shown to increase the infectivity of pandemic H1N1 influenza virus (pH1N1) in small airway epithelial cells (SAECs). Our group has identified several mechanisms driving SWNT-induced viral infections including modulation of the influenza receptor, sialic acid (SA). SA participates in influenza entry into target cells. We designed experiments to investigate two questions: Do SWNTs alter the expression, localization, and function of SA?; Do different types of carbon nanotubes modulate the influenza receptors and viral infectivity similarly? To answer these questions, we characterized

the expression of two SA linkages α 2,3 and α 2,6 on SAECs relevant to pH1N1 infections. Using immunohistochemistry, qPCR, and flow cytometry, we determined that the α 2,3 linkage is more prevalent compared to the α2,6 linkage. Furthermore, expression levels of the sialyltransferase ST3GAL4, responsible for adding the α2,3 linkage, was greater than ST6GAL1 which adds α 2,6 linkages. The α 2,3 linkage is associated with lower respiratory tract epithelium and increases the virulence of influenza by transmission and secondary infections. These data provide evidence that SAECs are a good model system for studying changes in influenza receptors by nanotubes and pH1N1. To determine the impact of various nanotubes on viral infectivity, SA linkage localization, and siayltransferase expression, SAECs were exposed to SWNTs or multi-walled nanotubes (MWNTs) and subsequently infected with pH1N1. Infectivity was measured by a TCID50 titer assay. The results indicate MWNTs caused a dose dependent decrease in viral infectivity up to ten fold where SWNTs demonstrated increased infectivity five fold. SWNTs also caused a localization alteration of the α 2,6 SA linkage. We are currently determining whether similar patterns result during MWNT exposures. Overall, all carbon nanotubes do not have the same modulatory effect on viral entry and infectivity, which may be a result of changes in the α 2,3 SA linkage in SAECs. These studies further highlight a novel mechanism of nanoparticle toxicity unidentified to date and should be further investigated.

26. LIGHT PROGRAMS ACTIVATED CD8 T CELLS TO SURVIVE AND BECOME MEMORY CELLS IN RESPONSE TO A VIRAL PATHOGEN

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CD8 memory T cells play a critical role in protection against repeated exposure to respiratory viruses. Memory T cell development in the lung is still not fully understood in terms of the nature and source of the molecular signals that establish and maintain the memory state. LIGHT is a tumor necrosis factor family member expressed by immune cells such as activated T cells, dendritic cells, monocytes and granulocytes. It is known to interact with two receptors HVEM & LTβR, leading to differential outcomes. However, the role of LIGHT expressed specifically by CD8 T cells during respiratory viral infection is not known. Using adoptive transfer of LIGHT-deficient CD8 TCRtransgenic T cells responding to Ag in the context of respiratory vaccinia virus (VACV) infection, we found that LIGHT signaling in CD8 T cells had a small impact on the level of primary expansion, with at most 25-30% fewer CD8 T cells accumulating in the lungs at the peak of response to VACV infection. In striking contrast, very few effector CD8 T cells that lacked LIGHT survived the contraction phase to differentiate into long-lived memory cells in the lungs. Furthermore, we found that HVEM, but not LTBR, functions as a trans-activating binding partner for LIGHT expressed on CD8 T cells. These results underscore the importance of LIGHT in the memory CD8 T cell development and suggest that targeting LIGHT-HVEM interaction will help in designing CD8 T cell based vaccines against respiratory viruses.

27. PAPER-BASED MICROFLUIDIC DEVICES FOR DETECTING RNA FROM FLU VIRUS

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Point-of-care (POC) tests have the ability to deliver quick result directly at the site of analysis. Also, they can be carried out by personnel without laboratory medical training. These advantages make POC tests appropriate for preventing and controlling the epidemic outbreak of infectious disease. Conventional methods to detect pathogenic bacteria and virus, including plaque culture, enzyme-linked immunosorbent assay and polymerase chain reaction, are generally not practical for POC test. A miniaturized analytical device, however, offers many advantages over the conventional methods. Tests performed by micro devices include fewer and simpler manual steps, thus are accessible to unexperienced medical personnel and even patients themselves. Paper-based analytical devices (PADs) do not need a pump to operate, require small sample volume and are intrinsically cheap. Our group has developed laminated paper-based analytical device (LPAD) for detection of proteins, glucose and cotinine in synthetic urine samples. We have been working on a POC device for the detection of low concentration of nucleic acid from viruses. The LPAD was fabricated by cutting chromatography paper/nucleic acid extracting FTA cards, followed by laminating with films. 3D printed connecter/sealer clamps were made for connecting RNA extraction/amplification unit with absorbing unit as well as sealing the device for isothermal

amplification. Reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) was developed for detecting RNA from H1N1 virus. The LPAD is shown in Figure 1a. This device contains the RNA sensing pad sealed with films except for two tongues for sample loading and connection. Gel electrophoresis (figure1b) confirms the RTLAMP system for detecting RNA from down to 7 pfu in each 25 μL tube. Our experiments have shown that the successfully functioning of LPAD. The colorimetric detection of flu virus and its integration with LPAD system is under development.

28. SEROLOGIC EVIDENCE OF EXPOSURE TO INFLUENZA D VIRUS AMONG PERSONS WITH OCCUPATIONAL CONTACT WITH CATTLE

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Background: Influenza D virus (IDV), a novel influenza virus with proposed classification: family Orthomyxoviridae, genus Influenzavirus D, species Influenza D virus, has been associated with influenza-like illness in cattle and swine. More recently, anti-IDV antibodies have also been detected in other small ruminants. A seroprevalence of approximately 1.3% has been estimated for the general population.

Objectives: A serological study was performed to gain insight on the zoonotic potential of IDV transmission to human adults with occupational exposure to cattle in Florida.

Study: Human serum samples from 49 cattle-exposed and 11 non-exposed controls were screened for IDV antibodies using hemagglutination inhibition and microneutralization assays.

Results: A seroprevalence of 94% was detected among individuals working with young cattle in Florida.

Conclusions: Detection of a high seroprevalence (94%) suggests that IDV poses a zoonotic risk to cattle-exposed workers. Whereas it is still unknown whether IDV causes disease in humans, our studies indicate that the virus may be an emerging pathogen among cattleworkers.

29. SINGLE-WALLED CARBON NANOTUBES SUPPRESS PULMONARY IMMUNE RESPONSE AND INCREASE VIRAL INFECTIVITY ON INFLUENZA VIRUS EXPOSED MICE

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Background: Extensive application of nanomaterials has raised concern regarding their potential health impacts. Many toxicological studies have focused on injury caused by single nanoparticle exposures but few have investigated how such exposures can impact a host's immune response to pathogen challenges. Our previous work has shown that pre-exposure of lung cells to single-walled carbon nanotubes (SWNT) modulates expression of several inflammatory and anti-viral genes in concert with increased viral titers following subsequent exposure to influenza virus (IAV). To further investigate if this observation would occur in vivo, we performed experiments in a mouse model of dual SWNT-IAV exposure.

Methods: C57BL/6 male mice were randomly assigned into control, SWNT, IAV, and SWNT+IAV groups and exposed to either 20 ug of SWNT or control vehicle (pluronic) by pharyngeal aspiration on day 0. On day 3, animals were then given 3.4× 【10】 ^4 IAV or PBS by intranasal instillation. All animals were euthanized on day 7 and whole lung images were captured using near-infrared fluorescence (NIRF) to track and localize SWNT in tissues. Viral titer, immune cells in the bronchoalveolar lavage fluid (BALF), and expression of several immune genes in lung tissues were quantified.

Results: NIRF images suggest that SWNT remain in the lung over the course of the experiment. Results from viral titer assays show a 64-fold increase in the lungs of SWNT+IAV exposed mice compared to the IAV only group. Quantitation of immune cells in BALF indicated an increase in lymphocytes in the SWNT group, an increase in neutrophils in IAV group and a combined additive profile in the

SWNT+IAV group. Lastly, all IAV exposed animals showed increased expression of IFN β 1, IFIT2, IFIT3, CCL5, IL8 genes and interestingly, their expression was repressed in the presence of SWNT (SWNT+ IAV group) with the greatest effect occurring for IFN β 1.

Conclusion: These results demonstrate that SWNT can suppress the immune response in vivo that may lead to increased susceptibility to viral infections.

30. THE GLOBAL SPREAD OF MIDDLE EAST RESPIRATORY SYNDROME: AN ANALYSIS FUSING TRADITIOANL EPIDEMIOLOGICAL TRACING AND MOLECULAR PHYLODYNAMICS

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Since its discovery in 2012, over 1,600 confirmed cases of Middle East Respiratory Syndrome (MERS) have been documented worldwide and more than a third of those cases have died. While the greatest number of cases has occurred in Saudi Arabia, the recent export of MERS-coronavirus (MERS-CoV) to South Korea showed that a pandemic is a possibility that cannot be ignored. Due to the deficit of knowledge in transmission methodology, targeted

treatment, and possible vaccines, understanding this virus should be a priority.

There were three aims: (1) to examine case incidence trends over time and geographic area using epidemiological information, (2) to trace evolutionary history of the virus in circulation using genetic data, and (3) to explore ways in which these two analyses can be combined to design public health interventions. By performing a systematic review of literature on MERS-CoV, we performed a qualitative meta-analysis of all laboratory confirmed cases worldwide to date, with emphasis on international transmission and healthcare associated infections. In parallel, we used publicly available MERS-CoV genomes from GenBank to create a phylodynamic tree, detailing geospatial timeline of viral evolution.

The spatiotemporal history of MERS cases, as documented epidemiologically, was supported by the phylodynamic analysis, and in addition, we were able to calculate basic reproduction numbers for MERS-CoV in humans and camels whose difference can be insightful in designing public health interventions. By combining traditional epidemiological tracing with phylodynamics, we present a holistic picture of the MERS epidemic from molecular level to global scale.

31. VALIDATION OF INFLUENZA-LIKE-ILLNESS CATEGORY FOR SYNDROMIC SURVEILLANCE SYSTEM, ESSENCE, USING INFLUENZA LABORATORY TEST RESULTS

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Background: Syndromic surveillance is essential to the field of Public Health, particularly within the Epidemiology sector, as it assists in a multitude of issues including identifying changes in disease incidence and estimating the severity of disease. This study aims to investigate ESSENCE's ability to identify positive influenza rapid antigen test results through its influenza-like-illness syndrome category.

Methods: Influenza rapid antigen test results were collected from a local Polk County hospital (n= 1973). Data was used from four time periods—weeks 50 through 53 of 2014, and week 4 of 2015. An ILI syndrome query was performed in ESSENCE using the corresponding dates; only including data from the hospital in which tests results were provided. We analyzed the syndromic surveillance system data to assess its positive predictive value.

Results: The positive predictive value calculated for the syndromic surveillance system, ESSENCE, was 36%. The proportion of positive laboratory results to the number of influenza like illnesses identified in ESSENCE were 30%, 30%, 24%, and 5%--respective to the corresponding weeks. This proportion increased with the peak of the flu season, which approximates the overall PPV. Flu season peaked during weeks 51 and 52, and began to decrease at week 53 through week 4.

Conclusion: This study provides insight on the value of syndromic surveillance when coupled with laboratory testing. Although the system was proven to have a lower positive predictive value the

calculations show that the proportion of positive influenza results to the number of ILI identified in ESSENCE parallels the overall PPV.

32. A NEW MECHANISTIC MODEL TO SIMULATE EFFECTS OF DIURNAL TEMPERATURE OSCILLATIONS ON POTATO LATE BLIGHT DEVELOPMENT

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Global climate change is associated with increased average temperatures and changes in diurnal temperature ranges. Previously, it was shown that the optimum temperature curve for incubation and latency development rates of Phytophthora infestans on potato leaflets was steeper under constant (10-27°C) than under oscillating temperatures (with amplitudes of 5°C and 10°C) in a growth chamber. The optimum curves for lesion growth rate, infection efficiency and sporulation were increased under oscillating temperatures with amplitude of 5°C but decreased with amplitude of 10°C compared to constant temperatures. Using these results, a mechanistic model (BLIGHTSIM) was developed to simulate late blight under oscillating versus constant temperatures, and predict potential late blight outbreaks under climate change. BLIGHTSIM is a modified Susceptible (S), Latent (L), Infectious (I) and Removed (R) compartmental model with hourly temperature and relative humidity as input variables. The model was calibrated with data

obtained from growth chamber experiments and validated with field data from Ecuador. The model provided a good fit (R2 of regression of simulated on observed data) to both growth chamber and field data. The model revealed no significant interaction between average temperature and temperature amplitude with respect to the effects on Area Under the Disease Progress Curve (AUDPC). In addition, there was no effect of starting time on AUDPC when the model runs were initiated with latent sites, but the AUDPC was slightly greater when the model runs initiated with infectious sites started at 1.00 am compared to the other times of day. BLIGHTSIM will be incorporated in a potato growth model and used to study effects of changes in average temperature and diurnal oscillations on potato late blight.

33. ANTIFUNGAL POTENTIAL OF ANTIMICROBIAL PEPTIDE MIMETICS IN A MOUSE MODEL OF DISSEMINATED CANDIDIASIS

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Disseminated candidiasis is a life threatening fungal infection, associated with a high morbidity and mortality in immunocompromised patients; especially those caused by Candida albicans and other non-albicans Candida (NAC) species. Due to limited therapeutic options, unintended toxicity as well as emergence of resistant strains emphasizes the urgent need to develop novel antifungal drugs. Antimicrobial peptides (AMPs) are

small cationic peptides, are effective as broad-spectrum antibiotics and antifungals, and exhibit limited resistance development. However, high production cost and protease degradation are the main drawback of AMPs. Here we studied in vitro activity of eight nonpeptidic molecules that mimic the structure and function of AMPs, and demonstrated their efficacy in a mouse model of disseminated candidiasis. The aim of the study was to establish these AMP mimetics as potential agents to treat systemic candidiasis. We studied in vitro activity of these mimetics against C. albicans and five other NAC, in the presence and absence of human and mouse serum using an MIC assay. We then quantified their activity in 8 week old CD-1 Swiss-Webster male mice injected (i.p.) with 150 mg/kg cyclophosphamide prior to injecting (i.v.) 3.6 x 10,000 cfu, a defined mouse model of systemic candidiasis. All mimetics exhibited MIC values below 10 μg/ml against the Candida species, and low cytotoxicity as quantified by MTT assay in three human cell lines. All mimetics tested caused rapid fungal membrane permeability as measured by propidium iodide uptake using flow cytometry. A competitive inhibition of the mimetics by divalent cations was also observed. In the mouse model of systemic candidiasis, injection (s.g.) of several of the mimetics in 20% kleptose, 2 hr post-infection, resulted in a reduction of kidney burden at 24 hr post-infection with an efficacy that was comparable to fluconazole. Our data demonstrate that antimicrobial peptide mimetics are a potential source of antifungal agents that could be developed as novel therapies for systemic candidiasis.

34. EVOLUTIONARY RELATIONSHIPS AMONG PLANT AND ANIMAL OOMYCETE PATHOGENS BASED ON PHYLOGENOMIC ANALYSIS

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Oomycetes are fungal-like non-photosynthetic microorganisms that are evolutionary close to brown algae and diatoms, together forming the eukaryotic group named Heterokonta or the stramenopiles. There are two major lineages of Oomycetes: the Saprolegniomycetidae and Peronosporomycetidae. Most of the animal-infecting oomycetes are in the saprolegnialean lineage and most of the plant pathogens are in the peronosporalean lineage. Peronosporaleans include Phytophthora and Pythium species, downy mildew pathogens and white blister rusts. Phytophthora species, which means 'plant destroyer' in Greek, include some of the most devastating plant pathogens and includes the best-known oomycete: Ph. infestans, which causes late blight of potato and resulted in the great Irish famine in the 1840s. In the last six years, whole genome sequences have been generated for six Pythium species, plus ten other oomycetes. This important genomic resource has allow us to gather the largest number of coding sequence markers to report for the first time a phylogenomic analysis in Oomycetes, with a

particular focus on Pythium. In this study, we included for the first time the whole genome sequence of the unique animal pathogen Py. insidiosum. This species is the causal agent of pythiosis, a deadly disease of horses, dogs, cattle and other mammals, including humans, in tropical and subtropical regions.

We used the nucleotide sequences of 277 orthologous genes found in 17 oomycete genomes. Our phylogenomic approach showed Hyaloperonospora within the Phytophthora clade while Albugo was placed as a sister group of all the peronosporaleans taxa. We confirmed the clustering of Phytopythium vexans to the Phytophthora-Hyaloperonospora clade, which was previously suggested using sequences of the nuclear ribosomal gene and the mitochondrial COI. We found two monophyletic groups within Pythium. One group was composed of three taxa that correspond to Pythium clades A, B and C, respectively. The other group was composed of taxa representing clades F, G and I. These two groupings are consistent with previous Pythium phylogeny based on ITS and 5.8S ribosomal gene. However, Pythium clades F, G and I formed a sister group to the Phytophthora-Hyaloperonospora-Phytopythium, thus confirming the lack of monophyly of the genus Pythium sensu lato and highlighting the need for genomic, morphological and physiological studies of this group and all Oomycete as a whole.

35. GLOBAL POPULATION GENETIC DIVERSITY AND PHYLOGENETIC ANALYSIS OF PHYTOPHTHORA PALMIVORA

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The global spread of plant pathogens has been expedited by climate change, international travel and trade. Exotic pathogens have invaded regions with susceptible hosts and new damaging genotypes have been introduced to existing populations.

Phytophthora palmivora (Butler) is a destructive heterothallic oomycete that is commonly found throughout the tropics and subtropics. It infects a wide range of plant hosts and results in losses in fruit and vegetable crops. High genetic diversity was previously reported in isozymes and RAPDs genotyped in a collection of isolates from Southeast Asia, suggesting Southeast Asia as the center of origin for P. palmivora. However, the worldwide population structure and phylogeography of P. palmivora population have not been explored by gene sequencing methods.

A total of 94 representative isolates of P. palmivora from five continents (Africa, Asia, Australia, North America and South America) were investigated by multilocus sequence analysis. Our results showed that the origin of the global population of P. palmivora was Philippines and Indonesia in Southeast Asia. In contrast, the South America population played the role of a "bridgehead" for global colonization. Host jumps from coconut, native to Asia, to hosts native to South America, such as cocoa and rubber, appear to have

contributed to global epidemics of P. palmivora. Furthermore, noteworthy population subdivision was detected in our global sample of isolates. Our results suggest that wild relatives of P. palmivora hosts in Philippines and Indonesia may display resistance to P. palmivora and disease control may rely on blocking further movement of the pathogen from centers of diversity in Philippines, Indonesia and South America.

36. GLOBAL SPATIAL DISTRIBUTION OF BLUEBERRY TWIG BLIGHT (PHOMOPSIS VACCINII) PROJECTED BY TWO SPECIES DISTRIBUTION MODELS

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Blueberry twig blight, caused by Phomopsis vaccinii (teleomorph Diaporthe vaccinii), is a severe endemic disease in the Eastern and Northwestern USA. It has been found in Europe, Canada, Chile and China. In the latter two countries and most of Europe, the pathogen was eradicated, except for Latvia and Belarus. Publications on its occurrence in the USA and Canada indicate that this is a cool-season pathogen. Models on the potential geographical range of P. vaccinii

have not been published. Published data on worldwide occurrence were inventoried and supplemented with National Plant Diagnostic Network (NPDN) data in the USA. These occurrence data and longterm climate data from the Worldclim website were entered in the niche models MaxEnt and Multi-Model Framework. The models predicted that the climate in the central and eastern USA (including Florida) and the west coast of the USA and Canada would be conducive to blueberry twig blight. Large areas in Europe, eastern Australia and New Zealand, and smaller areas in South America and East Asia would be conducive too. All locations where twig blight had been identified were correctly predicted to be conducive. Precipitation in the driest quarter and mean annual temperature contributed most to the prediction. The models indicate that P. vaccinii is not limited to cool climates, although the optimal annual average temperature is 10°C according to the MaxEnt model. For the first time, the NPDN database was shown to be an important source of information for the prediction of the potential global spread of a plant pathogen.

37. INFECTIOUS ZOOSPORES OF PYTHIUM SPECIES SHOW ATTRACTION TO ANIMALS

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Infectious zoospores of Pythium species show attraction to animals

Pythium insidiosum is an oomycete pathogen uniquely able to invade and cause the disease pythiosis in animals. P. insidiosum affects horses, dogs, and humans, causes subcutaneous lesions, and can be lethal in mammals. Pythiosis is a relatively rare disease that mostly occurs in the tropics or subtropics but has recently been reported in California, Arizona, and Florida, suggesting that this organism is expanding its infectivity to other environments. The infection is acquired by mammals through small wounds that came into contact with zoospore-infested water. Zoospores are motile spores that are the primary infectious propagules. P. insidiosum is unusual in the genus Pythium in causing disease in animals rather than plants. It is important to determine why this species can infect mammals. Recently, researchers have questioned whether species closely related to P. insidiosum show some of the same traits as P. insidiosum. This study examines chemotactic attraction towards plant and animal-derived substances.

The purpose of this research was to determine if the zoospores of P. aphanidermatum (P1771), P. catenulatum (33H), and P. apleroticum (14H) exhibit chemoattraction to animal-based substances. Each culture was grown in V8 media and transferred to a CaCO3 solution for induction of zoosporulation. Zoospores of each isolate were induced within 24 to 48 hours. The zoospores of each species were

pipetted onto a glass slide chamber with capillary tubes containing plant and animal test substances in one tube and a water control in the other capillary tube. The glass chambers were incubated for 1 hour at room temperature and overnight at 5 degrees Celsius and were observed the following day under a compound microscope. The number of zoospores within 0.75 mm and 2 mm of each capillary tube was counted. SDB (Sabouraud dextrose broth) and other animal-derived substances attracted zoospores of all 3 species. This study establishes that other Pythium species do have the potential to colonize mammals.

38. LIVE RECOMBINANT ATTENUATED SALMONELLA VACCINES AGAINST MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis (TB) has been a significant cause of morbidity and mortality for the human race from prehistorical times to the present. The World Health Organization estimates that one-third of the world's population is infected with Mycobacterium tuberculosis, the causative agent of TB. In 2014, 9.6 million new cases of TB were

diagnosed and 1.5 million individuals with active disease died of TB, making TB once again the leading cause of death from a disease caused by a single infectious agent. In countries where AIDS is endemic, the diseases caused by HIV and M. tuberculosis act synergistically, with devastating effects on the infected individuals and on the societies in which they live. In addition, annual increases in the incidence of infections caused by multi-drug resistant M. tuberculosis strains make treatment increasingly difficult. Although there is a vaccine against M. tuberculosis (M. bovis BCG) that is used in some parts of the world to protect infants and young children from serious complications of TB, protection is not long-lasting and by the time individuals reach adolescence, they are fully susceptible to infection. Recombinant attenuated Salmonella vaccines (RASVs) offer an attractive alternative to BCG for delivering M. tuberculosis antigens to elicit better protective immunity. Orally administered RASVs are able to elicit mucosal, antibody and cell-mediated immune responses to antigens produced by the RASVs. We have constructed RASVs that display regulated delayed lysis and regulated delayed antigen synthesis following immunization. We have introduced plasmids with genes encoding three immunodominant antigens of M. tuberculosis (Early secreted antigenic target 6 kDa [ESAT-6], culture filtrate protein 10 [CFP-10] and Antigen 85A [Ag85A]) into the RASVs and used these RASV-Mtb constructs to orally immunize mice. Mice immunized with RASVs producing these three M. tuberculosis antigens have been protected as well or slightly better than mice immunized subcutaneously with BCG against aerosol infection with virulent M. tuberculosis. We have demonstrated that the RASV-Mtb vaccine elicits significant antibody and cellular immune responses that contribute to protection against M. tuberculosis infection. We continue to modify our RASV strains to improve their immunogenic potential and we are evaluating nine other candidate M. tuberculosis antigens for possible inclusion to further enhance our RASV-Mtb vaccine constructs.

39. MOXIFLOXACIN AND PYRAZINAMIDE DOSE OPTIMIZATION FOR MULTIDRUG RESISTANT AND EXTENSIVELY DRUG RESISTANT TUBERCULOSIS

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Introduction: The global emergence of multidrug-resistant tuberculosis (MDR TB) is an enormous public health threat and major barrier to effective TB control. In 2013, the World Health Organization (WHO) estimated 480,000 new cases of MDR TB worldwide and 210,000 MDR TB-related deaths, and stated we are "off track" in controlling the epidemic.

Hypothesis: We hypothesize that the concentrations of pyrazinamide (PZA) and moxifloxacin (MXF), a main fluoroquinolone for the treatment of MDR TB, are lower inside tuberculous lesions, the ultimate site of action, compared to serum. Lower drug concentrations in cavitary lesions may lead to development and amplification of resistance and ultimately to treatment failure. In addition, we assert that optimal drug concentrations will be associated with a faster time to culture conversion and less development of acquired drug resistance. Here, we test in vitro systems in support of ongoing clinical testing of lung penetration of drugs, based on microdialysis measurements.

Methods: We have developed an in-vitro system to evaluate the pharmacodynamic effect by performing time-kill curves, and to investigate a potential synergistic effect of the drugs. Furthermore, we investigated the possibility of simultaneous measurement of PZA and MXF using microdialysis.

Results: The minimum inhibitory concentration (MIC) for the single drugs MXF and PZA against the tuberculous strain H37Ra was established as 0.5 μ g/mL and 75 μ g/mL, respectively. With increasing concentrations of MXF, lower concentrations of PZA were required to produce no visible growth when combining both drugs. A time-kill curve for MXF against H37Ra was performed suggesting that the maximum pharmacodynamic effect is being reached at 2x MIC. In vitro, microdialysis recoveries were not altered when MXF and PZA were investigated simultaneously.

Discussion: PZA and MXF appeared to show additivity against TB strain H37Ra in vitro. Higher concentrations of MXF did not appear to enhance killing. Microdialysis testing in vitro supports the application of this tool for measuring PZA and MXF from surgically excised cavitary TB lesions.

40. A PILOT STUDY ON HUMAN SCHISTOSOMIASIS IN A RURAL COMMUNITY OF FIANGA, REPUBLIC OF CHAD

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Schistosomiasis, a water-associated parasitic disease and part of neglected tropical diseases (NTDs), still poses a significant public health threat in many parts of developing countries. In recent years there have been increasing national control programs against schistosomiasis and other NTDs. This was encouraged by the World Health Assembly resolution WHA 54.19, and the availability of a costeffective drug for the schistosomiasis morbidity control. Human schistosomiasis is an important public health problem in Cameroon and Chad but the control program (chemotherapy) is only implemented in Cameroon. Datcheka (Cameroon, Far-North Region) and Fianga (Chad, Mayo-Kebbi East Region), two neighboring subdivisions located across the border of the two countries, are populated by the same ethnics groups. People from both sides constantly move across the border and this can affect the epidemiology of schistosomiasis transmission in both sides. The aim of this study is to assess current schistosomiasis situation in villages in the Chadian side and how it might impact the situation in

Cameroon. Here we present preliminary results concerning the parasitological survey and environmental characterization on potential transmission sites for schistosomiasis. This study was conducted in December 2014 and five schools were selected in Fianga, based on the geographic localisation and the presence of environmental risk factors for schistosomiasis. In each school, 50 schools children were randomly selected to participate to the survey. Upon receiving approval of parents and local authorities in charge of Health and Education, selected children were requested to provide stool and urine specimens which were examined using the Kato-Katz and sedimentation techniques, respectively. Overall, only Schistosoma haematobium eggs were found in urine samples with a prevalence rate of infection of 53.4% ±0.5 and an average of 13±23 eggs/20µl of sediment. Prevalence of infections in Kiriou, Tchangbele and Deheing were ≥ 50% whereas in Gabra and Kaski the prevalence of infections were between 10% and 49%. From our investigation we observed that villages were very poor and relied mostly on temporary water bodies for their daily activities, with an exception for Tchangbele situated beside an artificial lake (lake Tikem). In kiriou, population create natural reservoirs to retain rainfall water during rainy period to help cultivation during the dry season. Schistosoma haematobium infection is a real public health problem in Fianga and poor life and environmental condition seem to be important factors to be considered for future control plan.

41. ASSESSMENT OF THE DISTRIBUTION AND OCCURRENCE OF DIARRHEAL PATHOGENS OTHER THAN VIBRIO CHOLERAE IN A HAITIAN POPULATION

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Globally, diarrheal disease affects more than 1.7 billion individuals each year and ranks it as the second leading cause of death in children less than five years of age. While cholera received major attention in Haiti since 2010, the diarrheal disease burden caused by other microbial pathogens remains significant and has not been well documented in Haiti. Between June 2013 and May 2014, diarrheal stool samples were obtained from the patients who were admitted at different hospitals and clinics (other than the cholera treatment center) in semi-urban area in Haiti. Mostly, patients with severe diarrhea go to the cholera treatment center and samples from this setting have previously been analyzed and shown to possess a low level of other pathogens. Of 811 diarrheal stool samples examined, 454 (56%) contained one or more enteropathogens. The prevalence of major bacterial pathogens, included 79 enteroaggregative Escherichia coli (17.4%), 62 enteropathogenic Escherichia coli

(13.7%), 58 (12.8%) Salmonella non typhi serovar, 35 (7.7%) other Vibrio spp., 20 (4.4%) Vibrio cholerae O1, 18 (4.0%) enterotoxigenic Escherichia coli, 12 (2.6%) Shigella spp., 4 (0.9%) V. cholerae non O1/O139, 4 (0.9%) Aeromonas spp., and 1 (0.2%) Campylobacter spp. Parasites were also common with 55 (12.1%) Giardia spp., 2 (0.4%) Entamoeba histolytica, 1 (0.2%) and Cryptosporidium spp. Furthermore, we detected a total of 81 (17.8%) helminths that included 49 (10.8%) Ascaris spp., and 32 (7.0%) Trichuris trichiura. We also examined a subset of 200 (24.7%) samples for the detection of norovirus; out of 200 samples, 15 (7.5%) were positive for norovirus.

Our data indicate that a variety of diarrheal pathogens are responsible for enteric diseases among patients in Haiti with Enteroaggregative Escherichia coli is the most prevalent pathogen, followed by enteropathogenic Escherichia coli.

42. CAN PLANT EXTRACTS SERVE AS GOOD CANDIDATE MOSQUITO REPELLENTS AGAINST AEDES AEGYPTI?

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Aedes aegypti (L.) (Diptera: Culicidae) transmits viral pathogens to humans that can lead to severe human diseases, such as yellow fever and dengue fever. Environmental conditions, overcrowding, poor hygiene and healthcare resources, travel, and urbanization

contribute to the spread of infectious diseases. For example, malaria is a major health issue and a significant cause of morbidity and mortality in developing countries in Sub-Saharan Africa. One common approach for preventing and reducing mosquito bites is the use of repellents. DEET (N,N-diethyl-3-methylbenzamide) is the most common repellent on the market and is the active ingredient in sprays, aerosols, and lotions. The amount of DEET in these products ranges from 5 - 100%, and there are concerns about repetitious use of synthetic chemicals on the skin. Therefore, many researchers have been focusing efforts on the discovery of safe and effective repellents from natural sources. The cosmetic industry is using plant extracts in cosmetic products and an increasing number of plantbased therapeutic agents are available as complementary dermatologic therapies. Botanicals such as Rose, Matricaria, Arnica, Calendula, Mentha, Helichrysum, Lavandula, Rosemary, Ocimum, Tanacetum, and Achillea species are widely used in dermatological and cosmetic formulations. The objective of this study was to investigate the repellency of this group of botanicals against Ae. aegypti using a protocol that involves testing by human volunteers. For the selected essential oils, the range of repellency based on the minimum effective dosages (MED) for repellency was 0.078 - 0.375 mg/cm2. For reference, the MED for DEET was 0.039 ± 0.014 mg/cm2. Ocimum essential oils were repellent, with MEDs ranging from 0.063 - 0.750 mg/cm2 in 43 different Ocimum essential oils. Tests done from the n-hexane and ethyl acetate extracts, as well as essential oils of the German and Roman chamomile flowers, indicate that the German chamomile essential oils were the most repellent (MEDs of 0.047-0.188 mg/cm2). The Roman essential oils had MEDs of 0.250 to 0.281 mg/cm2, while the n-hexane and ethyl acetate extracts were not repellent up to 1.5 mg/cm2. The positive control, DEET, displayed repellency between 0.006 to 0.039 mg/cm2 in our assays. The samples from Arnica, Calendula, Mentha, Helichrysum, Lavandula, Rosemary, Tanacetum, and Achillea are currently being

extracted and will be tested in laboratory assays. The chemical composition of rose, Ocimum, and Matricaria samples are currently being analyzed and the chemical constituents from these samples could lead to the further development of new repellents from natural sources.

43. CHARACTERIZATION OF DIFFERENT AEDES AEGYPTI POPULATIONS IN FLORIDA FOR INTERFERING WITH CHIKUNGUNYA VIRUS TRANSMISSION

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Our ability to assess risk and pre-vent mosquito-borne disease in Florida is hindered by lack of knowledge about mechanistic causes of differential vector competence. We need to understand vector-virus interactions that will lead to practical approaches for rapid risk assessment of disease in Florida.

We evaluated the vector competence of Florida Aedes aegypti for chikungunya virus (CHIKV) and determined a geographic component that influences genes involved in CHIKV competence. Results from these studies suggest that the three mosquito populations used in the study can become infected with the La Reunion strain of CHIKV, with infection rates well above 50%. The Rockefeller strain of Ae. aegypti had a dissemination rate of only 41%, suggesting that mosquitoes from this population are not effective vectors. Dissemination rates for Key West and Vero Beach populations were 80 and 100%, respectively, suggesting both are competent vectors

and might be involved in transmission. Infection and dissemination rates appeared to be influenced by geographic region.

Results from RNA sequencing were used to detect genes that interact with CHIKV and potentially used to interfere with infection. Understanding vector virus interactions in natural populations of Ae. aegypti can assist in development of an early warning of local CHIKV transmission.

44. CHARACTERIZING THE PYRETHROID RESISTANCE OF TWO STRAINS OF ANOPHELES GAMBIAE TO PERMETHRIN AND ETOFENPROX

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The African malaria mosquito, Anopheles gambiae, is the primary vector of malaria in sub-Saharan Africa, and is responsible for the disproportionately high burden of global malaria. Despite research in malaria vaccine development and pharmaceuticals to treat the malarial parasite; chemical insecticides remain the major source for decreasing malaria by killing the mosquito vector. In particular, pyrethroid insecticides are commonly used to control mosquito populations, and therefore disrupt the transmission of mosquito-borne diseases. However, the widespread use of chemical insecticides has predictably resulted in mosquitoes developing insecticide resistance. Two common types of insecticide resistance are an increase in the Phase-I and Phase-II metabolic systems, and target site modification. Knockdown resistance (kdr) is the reduced sensitivity of the nervous system caused by point mutation(s) in the insect voltage-sensitive sodium channel. The presented research will

focus on characterizing two An. gambiae strains, G3 (pyrethroidsusceptible) and Akron-kdr (pyrethroid-resistant). These two strains were examined at two life stages; forth-instar larvae and adults. Etofenprox, which is sometimes referred to as a "pseudopyrethroid," is of particular interest to see if it could be used to control pyrethroid-resistant An. gambiae. The Akron-kdr strain had a resistance ratio of 150 and 39 for permethrin and etofenprox, respectively, to forth-instar An. gambiae larvae at 24-hr. In adult An. gambiae, the Akron-kdr strain had a resistance ratio of 11 and 0.6 for permethrin and etofenprox, respectively at 24-hr. These toxicological studies indicate that An. gambiae resistance in the Akron-kdr strain is more evident with permethrin versus etofenprox at both of the life stages tested. Additionally, a co-treatment of piperonyl butoxide (PBO) did not synergize the toxicity of etofenprox in either the susceptible or resistant An. gambiae strains; indicating that etofenprox is probably not effected by Phase-I oxidative metabolism. PBO moderately synergized permethrin in the Akron-kdr strain (synergistic ratio of 1.7). Current studies are underway to further characterize the mechanism of resistance amongst these two strains of An. gambiae.

45. FIRST OCCURRENCE OF DIAPHORINA CITRI IN EAST AFRICA, CHARACTERIZATION OF THE CA. LIBERIBACTER SPECIES CAUSING HUANGLONGBING (HLB) IN TANZANIA, AND POTENTIAL FURTHER SPREAD OF D. CITRI AND HLB IN AFRICA AND EUROPE.

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Citrus surveys were conducted at high (>700m), medium (300-600m) and low (<200m) altitudes in Tanzania. Adults and nymphs of Trioza erytreae (del Guercio) were abundant in the highlands and less abundant at medium altitudes. Unexpectedly, adults and nymphs of Diaphorina citri Kuwayama, the Asian citrus psyllid, were found and collected at medium altitudes, around Morogoro. No psyllids were observed at low altitudes. Severe huanglongbing symptoms and tree decline were evident at high altitudes, while mild and few symptoms were observed at intermediate and low altitudes, respectively. DNA

was extracted from leaf and psyllid samples and subjected to conventional PCR (cPCR) with seven different primer sets and RT qPCR with two primer sets. cPCR bands were sequenced and subjected to phylogenetic analysis. Candidatus Liberibacter africanus (Laf) was detected in highland leaf and T. erytreae samples from high and medium altitudes by all methods. Sequences from leaves and psyllids were similar to those from South Africa. Candidatus Liberibacter asiaticus (Las) was detected by qPCR in medium altitude leaf samples, but cross-reaction with Laf was likely because presence of Las was not confirmed by cPCR and sequencing. Neither Laf nor Las were detected in D. citri samples. This is the first report of D. citri in Africa and perhaps Las in Tanzania. Predictions were made of the potential distribution of D. citri and Las in Africa and along the Mediterranean coast using the correlative models MAXENT and Multi-Model Framework. Additional surveys at medium and low altitudes and guarantine measures are recommended.

46. HIGH THROUGHPUT ASSAY FOR TRANSMISSION OF ARBOVIRUSES BY MOSQUITOES

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Vector competence refers to the ability of a vector to acquire, maintain, and transmit an infectious organism. Mosquito transmission of an arbovirus by bite requires being susceptible to infection and permissive to replication and virus dissemination to the salivary glands. Collecting mosquito saliva in medium-filled capillary

tubes has become the standard for assessing arbovirus transmission. However, this method is time consuming and labor intensive, usually requiring multiple lab personnel for sufficient sampling. Here we compare the capillary tube method to an alternative high throughput approach; the collection of saliva on filter paper. We tested whether saliva could be collected on filter paper and reliably tested for the presence of arboviruses using a model system (Aedes aegypti, Aedes albopictus, and Chikungunya virus). Mosquitoes were allowed to feed on Chikungunya virus infected blood and tested for the presence of Chikungunya virus RNA by gRT-PCR in the saliva after five and 13 days using both methods. Individual mosquitoes were held in tubes and presented with honey-soaked filter paper. The honey was dyed with blue food coloring which provided a visual marker (mosquito midgut) indicating that a mosquito fed on the honey and expectorated saliva and virus. Transmission measurements using the collection of saliva and Chikungunya virus on filter paper were similar to or underestimated (4-18%) measurements by the capillary tube method. The filter paper method was comparable to the capillary tube method and is less labor intensive so that greater samples can be tested in a shorter period of time. Additionally, the filter paper method is a nondestructive approach so that the same mosquito can be tested multiple times following ingestion of arbovirus infected blood.

47. IDENTIFYING MALARIA RISK FACTORS IN A HYPER-ENDEMIC SETTING USING BAYESIAN MODEL SELECTION

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The epidemiological dynamics of malaria, as with many other vectorborne diseases, have been linked to a wide variety of environmental, socio-economic, and demographic factors. Traditional approaches for evaluating the contribution of each of these potential drivers present a tradeoff in research design. Modeling all possible combinations can be computationally intensive and make it difficult to draw definitive conclusions. Conversely, selecting a subset of potential drivers makes it impossible to observe the relative importance of a particular covariate and can exclude important risk factors. Here we propose a Bayesian probit regression model that contains a model selection procedure based on proposing a new candidate model from a subset of covariates pool through the random addition, subtraction, or swap of a parameter. A new model is proposed and evaluated at each step of the iterative Markov Chain Monte Carlo algorithm, generating parameter estimates and inclusion frequency for each potential disease driver. We used this approach to evaluate the relative importance of forty potential malaria risk factors in the Bunkpurugu-Yunyoo district of northern Ghana. Our analysis identified substantial protective factors related to the two modest "urban" centers over this small geographic area. This technique offers a promising solution for dealing with computational constraints of evaluating many risk factors for malaria and other diseases.

48. MAGNET: MALARIA GENOME EXPLORATION TOOL. INTEGRATED VISUALIZATION OF FUNCTIONAL GENOMIC DATA FOR PLASMODIUM 3D7

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The Malaria Genome Exploration Tool is a Java tool for the integrated visualisation of functional genomics data relating to the malaria parasite, Plasmodium falciparum and related species.

MaGnET is a 'workbench' style tool for browsing selected functional genomics datasets, which aims to reduce the sense of 'data overload' sometimes faced by researchers when trying to extract meaningful information from large-scale datasets. MaGnET allows users to select groups of genes and transport these groups across different datatypes to examine their relationships. The goal of this type of exploration would be to extract clues as to the function and biological process in which previously uncharacterised genes are associated and to formulate experimentally testable hypotheses.

49. MALARIA MORTALITY IN ZAMBIA

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Background: In 2014, there were roughly 438000 malaria deaths, globally, and Sub-Saharan Africa was home to 91% of them. Although Zambia has one of the highest malaria burdens worldwide, very little is known about malaria mortality there. In this study, we estimated the burden of malaria mortality and investigated the spatio-temporal trends of cases. We also measured the relationship amongst the existence of health facilities, coverage of indoor residual spraying (IRS) in houses and availability of long-lasting insecticide-treated nets (LLIN) to malaria mortality in Zambia.

Method: Annually reported, district-level, aggregated malaria mortality data from 1999 to 2014 were obtained from the Zambian District Health Information System. A district-level, random-effects model with negative binomial regression model was used to explore the association between malaria mortality and coverage with LLINs, IRS, access to roads, and railways, proximity to water bodies, and existence of health facilities. Malaria mortality was mapped to visualize spatiotemporal trends and detect hotspots.

Results: In total 116,167 deaths were reported from 1999 to 2014. The mortality rate among young children (5 years, 45% deaths). The average case fatality rate was 136 per 100,000 cases. There was a peak of deaths in 1999, followed by a declining trend until 2007 when the number of deaths started to increase through 2010. No clear mortality trend was observed in subsequent years. From 1999-2014, 36.7 million LLINs were distributed and 7.73 million houses were sprayed with insecticide. Malaria mortality was

heterogeneously distributed in Zambia. Persistent hotspots of high mortality (mortality rate was 50% in these areas) were scattered in districts across the country. IRS and LLINs were associated with reduced deaths in all age groups.

Conclusion: Targeted interventions in districts with persistent hotspots may help reduce malaria related mortality in Zambia.

50. MIGRATION AND MALARIA TRANSMISSION IN ARCTIC-BREEDING SHOREBIRDS OF NORTH AMERICA

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Migratory birds utilize a wide geographic range throughout their annual cycle which exposes them to diverse habitats and their associated pathogens. Hence, migration may facilitate the spread of emerging infectious diseases (EID) causing concern for public health and the conservation of the avifauna. Avian malaria is an EID with a nearly global distribution caused by protozoan blood parasites in three genera (Haemoproteus, Plasmodium, Leucocytozoon) that are transmitted via biting diptera. The migration-facilitated movement of avian malarial parasites by Arctic-breeding shorebirds remains poorly understood due to limited efforts in broad spatio-temporal sampling. Here, we evaluate prevalence and phylogenetics of avian malarial parasites in shorebird communities across the Arctic. We obtained n=1930 blood samples from Alaskan and Canadian Arctic breeding grounds over a three-year period (2011-2013). All samples were processed for genetic analysis, and screened for malaria parasites utilizing primers that amplify a segment of cytochrome b gene on the mitochondrial genome. We sequenced a ~550 bp region

of cytochrome b and developed a phylogenetic hypothesis based on sequences we recovered and closely related lineages deposited in GenBank and MalAvi. We observed low infection prevalence (1.1% -2.6%) at Arctic breeding grounds during the 3-year study, a finding that is consistent with previous short-term studies and lends support to the migratory culling hypothesis. It indicates that individuals infected with malarial parasites may have lower survival rates than non-infected individuals during long-distance migration and hence would not significantly contribute to the movement of these parasites. However, phylogenetic analysis suggested infection with malarial parasites occurs during northward migration to breeding grounds since lineages recovered from Arctic-breeding shorebirds are most closely related to Plasmodium and Haemoproteus species in the Caribbean, Central America and Southern United States. We conclude that the pathogenic effects of malarial parasites may be too weak to affect survival of Arctic-breeding shorebirds during migration and shorebirds may contribute to the movement of avian malarial parasites.

51. MODELING ZIKA VIRUS TRANSMISSION IN COLOMBIA

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In May 2015, Zika virus started circulating in the Americas. Zika virus is an Arbovirus transmitted by Aedes mosquitoes and in the Americas mainly by Aedes aegypti. The most common symptoms of Zika virus disease are fever, rash, joint pain, and conjunctivitis. The illness was described to be mild with symptoms lasting from several days to a week. The outbreak in the western hemisphere started in Easter Island in Chile, then spread widely in Brazil and currently according to the Pan American Health Organization, twenty-one countries in the Americas have active transmission of Zika Virus.

The outbreak in Brazil led to reports of neurological symptoms like Guillain-Barre syndrome and pregnant women giving birth to babies with microcephaly and other birth defects.

Colombia is a dengue endemic country. Chikungunya started circulating in October 2014 and Zika virus in October 2015.

Around 15.000 Zika cases have been reported in all the country since this virus started circulating.

We model the transmission dynamics of Zika Virus and estimate the basic reproductive number (Ro) and other parameters based on epidemiological surveillance data to understand better the transmission of Zika Virus. We analyzed and compared the epidemic curves of daily cases of Zika reported to the local epidemiological surveillance system. The Basic reproductive number was estimated around 6 to 8. This explains why Zika Virus is spreading rapidly in Latin America and the Caribbean as we observed in the last months in Aedes aegypti infested areas and rapid interventions are needed to prevent new cases and complications.

52. MOSQUITO RESPONSE TO INDIRECT AND DIRECT EFFECTS OF PREDATION

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The use of biological control agents (e.g., predators, parasites) is a potential control strategy for an integrated past management approach to controlling emerging mosquito-borne diseases. Although the lethal effects of biological control interventions are well-characterized, the consequences of the non-lethal effects that may alter the potential for mosquitoes to transmit pathogens are less clear. We experimentally tested the roles of direct and the indirect impacts of the predatory mosquito Toxorhynchites rutilus on life history traits of the yellow fever mosquito Aedes aegypti prey. Treatments involved manipulating the presence of predator, presence of predator cues, density reduction that simulated predator consumption, and controls. Our results showed an overall significant impact of treatment on the life history traits of the prey populations. Treatments involving density reduction resulted in the highest growth and shortest development time among all groups. The presence of a predator or cues resulted in mosquito adult life spans that were less than or equal to those of other treatments. These results will be discussed in relation to the potential impacts on disease transmission.

53. NO EFFECTS OF PENICILLIN INJECTIONS FOR CITRUS HLB CONTROL ON CULTURABLE BACTERIA IN PETIOLES AND RHIZOSPHERE, PENICILLIN RESISTANCE, AND PHYTOPHTHORA ROOT ROT

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Trunk injection with penicillin has been tested to control citrus huanglongbing (HLB), but environmental safety must be assured before approval of penicillin injection in groves. We investigated potential effects of penicillin injection on rhizospheric and endophytic bacterial populations using grapefruit trees in field and greenhouse experiments. Trees were injected with penicillin G, and leaf and root concentrations were assessed in bioassays with Bacillus subtilis. Bacteria were isolated on a low carbon medium from roots plus rhizosphere and surface-sterilized petioles at various times after penicillin injection. Selected isolates were tested for penicillin resistance (20 ppm) and glyphosate resistance (7000 ppm), because glyphosate is widely used and cross-resistance against antibiotics had been documented. One month after penicillin injection half of the greenhouse trees were inoculated with Phytophthora nicotianae. Bacterial populations in petioles and root-rhizospheres initially

increased after penicillin injections, possibly due to release of nutrients from dead bacteria, then returned to control levels after one week. Penicillin resistance was common in isolates from penicillin-injected and control trees (30 to 94 %), and 62% of penicillin resistant isolates were resistant to glyphosate. Phytophthora root rot was not increased after penicillin injection. Thus, side effects of penicillin injection tested here were minimal.

54. PERFORMANCE OF THE ACCESSBIO CARESTART™ BIOSENSOR FOR THE DETERMINATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN RURAL HAITI PLAYING A CENTRAL ROLE IN MALARIA ELIMINATION

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common deficiency found in red blood cells and most who are affected remain asymptomatic unless exposed to oxidative stressors such as 8-aminoquinolines drugs (such as primaquine) at which time they can develop a hemolytic anemia. The prevalence of G6PD deficiency is high in the same parts of the world where malaria is also endemic, including Haiti. Because of primaquine's central role in the malaria elimination initiative and mass drug administration in Haiti, it is imperative to be able to identify those patients at risk for this hemolysis. Up until this point, the current gold standard and only quantitative test has been the spectrophotometer-based test which is not conducive to use in resource poor developing countries where

most cases of malaria are found. To increase the effectiveness in identifying individuals with G6PD deficiency, AccessBio Inc. has recently developed the CareStart G6PD Bioanalyzer which is a pointof-care quantitative test. This study assessed this new rapid quantitative test to the current gold standard test of spectrophotometry. We screened 346 school age children from three schools in Gressier, Haiti. G6PD levels were measured in the field using quantitative Biosensor test and then repeated in the controlled laboratory setting along with the Trinity Biotech spectrophotometer test. The Biosensor estimated the population prevalence of severe to moderate G6PD deficiency at 0.9 and 14.9%, compared to the gold standard estimates of 9.9 and 19.5%. The CareStart G6PD Bioanalyzer test had a high specificity (94.6% overall) and was able to determine G6PD negative population members. A recalibrated CareStartTM G6PD Biosensor showed improved correlation with the "gold standard" test (R2 = .7008). Specificity of 99.6% and the negative predictive value of 90.6% indicates usefulness in identifying those at risk of drug induced hemolysis (<30% residual activity).

55. POST-CHIKUNGUNYA FEVER EPIDEMIC CLUSTER OF DENGUE VIRUS 1 INFECTIONS AMONG SCHOOL CHILDREN IN GRESSIER REGION, OUEST DEPARTMENT, HAITI

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Dengue fever (DF) is the most common tropical and subtropical mosquito-borne viral illness. It is caused by four distinct Dengue virus (DENV) serotypes (1-4). In May 2014, an outbreak of Chikungunya fever (CF) caused by Chikungunya virus (CHIKV) started in Ouest Department, Haiti, and the ensuing epidemic spread throughout the country. As the epidemic waned in the fall of 2014, febrile illnesses among a cohort of school children in the Gressier region remained relatively high. One hundred seventy seven (n = 177) febrile cases from September 2014 to February 2015 that were suspected to be due to CHIKV infections were tested for CHIKV and all four DENV serotypes using RT-PCR and cell culture. Fourteen percent (25/177) were positive for DENV-1 virus by RT-PCR, none were positive for CHIKV, and 36/177 (20%) for DENV-4. The results indicate a common misdiagnosis of CHIKV, and active back to back transmission of DENV in this region of Haiti. Findings for DENV-1 are emphasized in this poster.

56. PREDICTORS OF EPIZOOTIC HEMORRHAGIC DISEASE SEROPOSITIVITY IN CAPTIVE WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)

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Hemorrhagic disease, or HD, is the most significant viral infection of white-tailed deer. The disease is caused by two closely related, but distinct, vector-transmitted viruses: epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV). In areas where these viruses are endemic, such as the southeastern United States, seropositive status for either virus may reach 90%. On Florida deer farms where animals are raised for meat and/or recreational hunting opportunities, high mortality epizootic events occur frequently but the reason why is unknown.

We investigated the relationship of EHDV exposure for each of the three serotypes of the virus found in Florida (EDHV-1, EHDV-2 & EHDV-6) with characteristics of captive deer from a farm in Gadsden County using generalized linear models (GLM; binary response variable: 1=seropositive, 0=seronegative). The most parsimonious model was selected using Akaike's Information Criterion (AIC). To confirm the findings of GLM we subsequently performed contingency table analysis and classification and regression tree analysis (CART).

In 2015, 60.9% of deer sampled were seropositive for EDHV-2, 44.9% for BTV, 43.5% for EHDV-6 and 36.2% for EHDV-1. The most parsimonious model for EHDV-1 seropositive status in captive deer included sex and exposure to the other EHDV serotypes. The most parsimonious model for EHDV-2 seropositive status in captive deer included age and exposure to EHDV type 6. Seropositive animals tended to be older (>1 year) and tended to be positive for EHDV-6. These findings suggest that repeated exposure to more than 1 serotype of HD virus occurs in captive white-tailed deer.

57. PREVALENCE AND PHYLOGENY OF AVIAN TRYPANOSOMA IN THE LOWVELD OF SWAZILAND, AFRICA

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Low lying areas have a higher mosquito presence and thus a higher incidence of vector-borne disease than neighboring areas. This connection is true for the Swaziland Lowveld which reports the highest incidence of human malaria in the country. Very little is known about vector transmitted diseases in this region, where irrigated crops have the potential to exacerbate the transmission of vector-borne disease. Trypanosoma is a widespread blood parasite causing multiple diseases including Chagas' disease and African sleeping sickness. Avian trypanosoma phylogeny is poorly developed but many species are believed to exist. We determined the prevalence of avian trypanosoma in ground-foraging birds and conducted a phylogenetic analysis. We trapped 32 Crested Guineafowl (Guttera pucherani) and 56 Village Weaver (Ploceus

cucullatus) ground-foraging birds in the lowveld of Swaziland with walk-in traps and collected 40ul of blood in 1ml of RNALater. We focused on Crested Guineafowl and Village Weaver because of large sample sizes and different ecologies. Crested Guineafowl are relatively large, ground-dwelling birds, whereas, Village Weavers are relatively small, arboreal-dwelling birds that forage on the ground. Both species are social birds, a potential risk factor for both species. DNA was extracted using Qiagen DNeasy Blood and Tissue Extraction Kits. Samples were screened for Trypanosomes using Valkiūnas et al. (2011) outer primers Tryp 763 and Tryp 1016 and inner primers Tryp 99 and Tryp 957 for a nested PCR, isolating a 770 bp 18s rRNA fragment. PCR product was confirmed on agarose gel and positive samples were submitted for sequencing. 26 of the 32 (81.25%) Crested Guineafowl and 42 of the 56 (75%) Village Weavers were positive for avian Trypanosoma. 40 sequences were recovered and used to construct a minimum spanning tree. This tree indicated that 23 of the 40 sequences are grouped into two clades and with no overlap of avian host between the two Trypanosoma clades. Although the two clades are not different species, this tree indicates that there may be some host species selection occurring in avian trypanosoma in the Lowveld of Swaziland.

58. PYRETHROID RESISTANCE AND BLOOD-FEEDING BEHAVIOR IN PUERTO RICAN AEDES AEGYPTI

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Emerging insecticide resistance is a major issue for vector control, especially in disease endemic areas. Resistance is associated with a higher cost in order to achieve a comparable level of chemical control, and has the potential to result in disease resurgence. Pyrethroid resistance has previously been documented in Puerto Rican populations of Aedes aegypti mosquitoes. In a related prior study, topical resistance to four insecticides was determined for susceptible (Orlando - ORL) and resistant (Puerto Rico - PR) strains of Aedes aegypti. Resistance ratios were calculated using the LD50 values, and the high resistance ratios calculated for permethrin (81) and etofenprox (243) support previous documentation of resistance in Puerto Rican mosquito populations. In the present study, behavioral differences in blood-feeding activity for pyrethroid resistant and pyrethroid susceptible strains of Aedes aegypti when exposed to pyrethroid-treated cloth were explored. In order to observe the blood-feeding behavior of both pyrethroid-resistant and susceptible laboratory colonies of mosquitoes, these populations were exposed to different concentrations of pyrethroid-treated uniform fabric in a stock cage filled with approximately 50 female Aedes aegypti mosquitoes for a 15 minute test. An untreated control sleeve of the same fabric type was paired with each treated uniform sleeve, in order to have a proper basis of comparison, since many uniform fabrics have different weave tightness which

determines how easily a mosquito proboscis can penetrate the material. Percent bite protection was calculated by utilizing Abbott's formula [{(C-T)/C}*100], where C = the number of mosquitoes bloodfed on a control uniform sleeve, and T = the number of mosquitoes blood-fed on a treated uniform sleeve. Results from these assays showed a shift in the dose-response curves for blood-feeding that indicated higher concentrations of pyrethroids were necessary to deter blood-feeding behavior in the pyrethroid-resistant Puerto Rican strain of Aedes aegypti. Interestingly, the resistance ratios for blood-feeding were similar for permethrin and etofenprox, but were lower than their respective resistance ratios for topical toxicity. This research is ongoing and future work will examine landing behavior and tarsal electrophysiology with pyrethroid-treated fabric.

59. RISK OF TRANSMISSION OF AN EMERGENT LINEAGE OF CHIKUNGUNYA VIRUS BY FLORIDA MOSQUITO VECTORS

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With epidemic chikungunya fever raging in the Americas, and the numbers of infected travelers arriving in Florida increasing, local transmission of this mosquito-borne virus is regarded as inevitable. Here we examine the emergence potential of chikungunya virus (CHIKV) in Florida by examining the vector competence of local populations of the two potential vector species, Aedes aegypti and Aedes albopictus. We tested whether Ae. aegypti and Ae. albopictus from Florida have a salivary gland barrier that limits CHIKV

transmission. We show that transmission of CHIKV is inhibited at low viremias by susceptibility to initial infection in the mosquito. At high viremias we found that Ae. aegypti and Ae. albopictus had similar and rapid rates of disseminated infections (18-65% by 2 days and 65-95% by 5-13 days since exposure), but differed in aspects of their ability to transmit CHIKV. The viral titer in the saliva of Ae. aegypti was 3-fold greater than Ae. albopictus and transmission efficiency for Ae. aegypti was greater than Ae. albopictus during the later stages of infection. We found no evidence to suggest differences in infection and transmission efficacies among various geographic locations in Florida for Ae. aegypti and Ae. albopictus. Rates of disseminated infections were similar for Florida Aedes and Ae. aegypti from the Dominican republic. However, Ae. aegypti from the Dominican republic had much lower rates of transmission than Florida Aedes, suggesting a more effective salivary gland barrier(s) in Ae. aegypti from the Dominican republic. The increased number of imported human cases of chikungunya fever in Florida poses a potential public health risk. Florida Aedes are highly susceptibility to infection and transmission of CHIKV after exposure to high viremias with declines in their ability to transmit CHIKV later during the infection.

60. ROLE OF HOST COMPOSITION ON THE TRANSMISSION DYNAMICS OF JAPANESE ENCEPHALITIS VIRUS IN BANGLADESH

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Japanese encephalitis (JE), a mosquito-borne zoonosis, is an important cause of viral encephalitis in eastern and southern Asia. The estimated incidence of JE in Rangpur Division is high in comparison to neighboring countries and JE was responsible for approximately 30% of all hospitalizations due to encephalitis in this region in 2014. Identifying which host species drive transmission could inform targeted interventions, such as focusing human vaccination campaigns on geographic areas with high transmission potential, and may suggest ways to reduce transmission among amplifying hosts. Since human infection does not contribute to the spread of disease and the human vaccine does not reduce transmission of JEV in the reservoir community, no herd immunity is generated and vaccination has to be sustained indefinitely. Mathematical models were used to predict where transmission potential for JEV is highest throughout Rangpur Division, based on the composition of domestic animal hosts within a village, abundance of mosquito vectors, and data from experimental infections of potential vertebrate hosts. Model predictions will be tested against data on seroconversion of sentinel animals and viral detection. If model-based estimates of transmission potential are a good indicator of JEV activity, this approach could be used to target high-risk areas for human vaccination or identify species for future interventions.

61. SPARSE SEROLOGICAL EVIDENCE OF PLASMODIUM VIVAX TRANSMISSION IN THE OUEST AND SUD-EST DEPARTMENTS OF HAITI

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Background: Plasmodium vivax infections, while quite prevalent throughout South and Central America, are virtually non-existent in Haiti, where P. falciparum infections are detected in over 99% of malaria cases. Historically, few cases of P. vivax have been reported in Haiti; all of which were identified by microscopy and none were confirmed by molecular diagnostics. To further examine the transmission of P. vivax in Haiti, a cross-sectional seroepidemiological study was conducted.

Methods: Whole blood was collected from 814 community members and school children ranging in age between 2 and 80 years-of-age from four locations in the Ouest and Sud-Est Departments of Haiti.

After separation of serum, samples were screened for antibodies toward P. vivax apical membrane antigen (AMA-1) and merozoite surface protein-119 (MSP-1) using an indirect enzyme-linked immunosorbent assay (ELISA).

Results: Of all participants screened, 4.42% (36/814) were seropositive for AMA-1, 4.55% (37/814) were seropositive for MSP-1, 7.99% (65/814) were seropositive to either antigen, and only 0.98% (7/814) were seropositive for both antigens. Seroconversion rates (SCR) for AMA-1, MSP-1, either AMA-1 or MSP-1, and for both AMA-1 and MSP-1 estimated from the cross-sectional seroprevalence indicated rates of P. vivax transmission of less than 1% per year.

Conclusion: Given the lack of historical evidence of P. vivax infections on the island of Hispaniola, the sparse serological evidence of antibodies toward P. vivax identified in the current study further support the notion that the transmission of P. vivax malaria might be extremely low or even completely absent in Haiti.

62. STUDIES ON THE MECHANISM OF ACTION OF MATRINE, AN INSECTICIDAL NATURAL PRODUCT

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As a tetracyclo-quinolizidine alkaloid, matrine is one of the bioactive components extracted from Sophora flavescens —a traditional Chinese medicinal herb that possesses strong antitumor activities through inhibition of cell proliferation and induction of apoptosis and is also known as a biopesticide with broad-spectrum effects against various insect pests, pathogenic fungi, bacteria and so on. While matrine is used as the main ingredient of a number of pesticides in China, the insecticidal activity against mosquito and the

mechanism of action at the tissue and cellular levels have not been documented. In order to facilitate the development of novel insecticides, we examined the action mechanism of matrine. Due to the water solubility of matrine, firstly, we studied the paralytic action in headless mosquito larvae using the method previous published by Islam and Bloomquist (2015). Groups of headless Aedes aegypti larvae exposed to matrine showed 50% paralysis at 7.6 ppm, with 95% fiducial limits of 4.5-10.2 ppm at the 5h time point. We also examined toxicity to adult Adeses aegypti. The results showed the LD50 was 258 ng/mg with 95% fiducial limits of 218-336 ng/mg and KT50 (time to 50% knockdown) was 27 min with fiducial limits of 25-29 min at the end of 1 hr at the LD50 dose. Thirdly, the effects of matrine on the evoked muscle EPSP of housefly were studied, based on published results that matrine affected ion currents in rodent heart muscle. The results demonstrated that little effect of matrine on EPSP amplitude or muscle membrane potential was observed. Interestingly, no EPSP block was observed approximately 640 seconds after 275 µM verapamil HCl (an L-type calcium channel blocker) was added to the bath, but there was complete block of the fast EPSP when combined with matrine at concentrations of 200 μM (N = 2). Therefore, matrine and verapamil appear to interact presynaptically to block the EPSP at micromolar concentration. Finally, experiments on the effect of matrine on the CNS of Drosophila melanogaster demonstrated that when the intact nerve sheath treated with 1-2 mM matrine, the nerve firing could be completely blocked for several minutes with little recovery, whereas matrine at micromolar concentrations only blocked the firing partially. Compared with 3-APS, a GABA(C)-receptor antagonist, both drugs gave similar results with subtle differences. Based on the above electrophysiological tests, it is concluded that the possible target of matrine in the CNS is the GABA receptor. For better validation, further cellular levels research need to be performed.

63. TICK-BORNE PATHOGENS OF CATTLE AND WILDLIFE IN SOUTH-CENTRAL FLORIDA

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Feral swine are known to harbor pathogens of humans and livestock such as Brucella suis and pseudorabies virus, and they may play a role in the transmission and distribution of tick-borne pathogens. Florida is estimated to have more than 500,000 feral swine, with the highest densities in south-central Florida, where the majority of cowcalf operations are located. In order to understand tick-borne pathogen exposure, prevalence, and transmission among cattle, swine, and deer, we collected blood samples and surveyed each mammalian species for ticks. Additionally, we collected host-seeking ticks throughout multiple habitats on a 10,000 acre working cattle ranch in South Central Florida. Beginning in May of 2015 and continuing to present, we have sampled > 70 feral swine, > 450 cattle, and six deer. Three genera of ticks were found attached to the animals (Amblyomma, Dermacentor, and Ixodes), and two genera of ticks were found while dragging (Amblyomma and Ixodes). Preliminary PCR results show the presence of Ehrlichia spp. in adult Amblyomma maculatum collected from deer and cattle. Serology results indicate that roughly 30% of cows surveyed have antibodies

to Anaplasma spp. Data collection and laboratory analyses are still in progress.

64. TOXICITY AND NEUROPHYSIOLOGICAL ACTION OF THE ISOXAZOLINE FLURALANER TO MOSQUITO AND FRUIT FLY

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Mosquitoes, such as Anopheles gambiae and Aedes aegypti, are important vectors transmitting mosquito-borne diseases. The first purpose of this research is to investigate the mosquitocidal activity of fluralaner; secondly, to confirm the mode of action of this new chemical class of GABA antagonist on Drosophila melanogaster larval central nervous system (CNS). In the first phrase of the study, the insecticidal activity of fluralaner (an oral veterinary parasiticide) was tested by bioassay. Fluralaner was toxic to Aedes aegypti mosquitoes in 24 hour exposures, with an LC50 (lethal concentration for 50% mortality) and LD50 (lethal dose for 50% mortality) values in larvae of 1.2 ppb and adult topical assays of 1.3 ng/mg. In adult topical assays, the LD50 value of fluralaner was 4-fold higher than fipronil, and 26-fold higher than permethrin. In time course studies, the insecticidal activity of fluralaner to adults increased by a factor of approximately two for each day of exposure, suggesting a slowly developing toxicity. The endpoint for fluralaner toxicity following topical application was about 5 days. In adult contact paper assays, a high concentration of 11 micrograms/cm2 of fluralaner could only kill around 12 % of the tested mosquitoes, which is much less activity than that observed on glass (LC50 = 14 ng/cm2). The data suggest that fluralaner does not have exceptional toxicity to mosquitoes in typical exposure paradigms. In electrophysiological recordings on Drosophila melanogaster larval CNS, 1 mM GABA was used to block nerve activity and the EC50 (effective concentration for 50% response) of fluralaner for restoring nerve firing (a measure of GABA antagonism) is 0.34 (0.06-1.8) μ M. The time to response of fluralaner was longer compared to dieldrin (6.3±5.8 minutes at 10 μ M), and in several preparations responses to fluralaner required 30 minutes or more to appear, suggesting a slow action even when the blood-brain barrier is disrupted. Although dieldrin (logP = 5.4) and fluralaner (logP = 5.01) have similar lipophilicities, the large size (mw = 556) of fluralaner compared to dieldrin (mw = 381) might influence its penetration through the mosquito cuticle and into the CNS.

65. TOXICITY AND PHYSIOLOGICAL IMPACT OF BASIC AMINES TO MOSQUITO AND COCKROACH

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The yellow fever mosquito, Aedes aegypti, a primary vector of dengue virus and other diseases, has been a major target of insecticide and repellent design and development. The basic amine, 1-methylpiperazine has been reported to incur an anosmic effect on Aedes aegypti so that the mosquitoes become incapable of detecting human odors. In the present work, lethal and electrophysiological actions of 1-methylpiperazine (pKa = 9.1) and the related basic amines, 1-methyl-pyrrolidine (pKa = 10.3) and triethylamine (pKa = 10.8, a commonly used anesthetic in the study of Drosophila melanogaster and mosquitoes) were investigated. Topical assays showed low toxicity of the amines, possibly because of vaporization off of the cuticle. 1-Methylpiperazine had a 1 hr KD50 of 4.7 (95%) fiducial limit 3.9-5.6, n= 3) µg/mg body weight that was not significantly different from the 24 h LD50 of 4.8 (3.5-6.1, n = 3)ug/mg body weight, suggesting low post exposure recovery. N-Methylpyrrolidine had 21% \pm 10.7% (mean \pm SD, n =3) mortality at 8 μ g/mg body weight, and trimethylamine had 3% ± 5.8% (mean ± SD, n = 3) mortality at the same dose. Vapor phase assays in glass tubes of 1-methylpiperazine, N-methylpyyrolidine and trimethylamine on Aedes showed instant knockdown followed by mortality at 15 μg/cm3, 55 μg/cm3 and 243 μg/cm3, respectively. All three basic amines increased the discharge frequency of mechanoreceptor neurons located in the American cockroach (Periplaneta americana) tarsus based on extracellular recordings of nerve firing patterns. When applied topically at the dose of 30 µg, nerve firing increased significantly compared to the acetone control group (ANOVA, Newman-Keuls multiple comparison, n = 5, p < 0.001). Patch clamp experiments showed inhibition of voltage gated potassium channels in the human neuroblastoma SY5Y cell line and a cell line expressing the Anopheles gambiae Kv2.1channel. N-methylpyrrolidine and 1methylpiperazine blocked potassium channels with IC50s in the micromolar range, with less inhibition of sodium channel current. Blockage of potassium current with little effect on sodium current is consistent with the neuroexcitation observed in cockroach leg neurons.

66. CHOLERA TRANSMISSION IN OUEST DEPARTMENT OF HAITI: DYNAMIC MODELING AND PREDICTION

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In this analysis is we present a comprehensive compartmental model for cholera transmission and apply it to the analysis of the data collected in Haiti. The challenge with cholera modeling, like with many other infectious diseases, is the course of the disease. The majority of the infected people remain asymptomatic and, therefore, unobserved. Underreporting is another issue. It is believed that external water reservoirs of bacteria and environmental factors temperature and precipitation play significant roles in triggering transmission and facilitating further spread of the epidemic. We propose the model that incorporates the incidence and the environmental data that are typically available from surveillance. When a new epidemic arises it is crucial to react appropriately. Public health measures such as vaccination campaigns can curb the epidemic or even stop it completely. We evaluate the effectiveness of the vaccination campaigns in our model. We also look at the behavior of the model over a period of ten years and evaluate the possibility of future outbreaks.

67. EFFECT OF SIMULATED MICROGRAVITY ON STREPTOCOCCUS MUTANS OXIDATIVE STRESS RESISTANCE, GLOBAL GENE EXPRESSION, AND BIOFILM FORMATION

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Microgravity can cause mandibular and alveolar bone loss and decreased saliva flow, host factors that could predispose astronauts to caries development and/or periodontal disease. However, the specific biological response of caries-causing oral bacteria such as Streptococcus mutans to microgravity has not been extensively investigated. In this study, High Aspect Ratio Vessel (HARV) S. mutans cultures were assessed for hydrogen peroxide resistance, competence, and for adherence to hydroxyapatite (HA) powder (simulating growth on tooth surface). Interestingly, S. mutans simulated microgravity cultures displayed increased killing by hydrogen peroxide challenge compared to normal-gravity cultures. However, the ability to uptake exogenous DNA was not affected. Although growth of S. mutans on HA was comparable between microgravity and normal gravity HARV cultures, increased planktonic growth was observed in the microgravity condition. Metabolomics and RNAseg analyses were also performed on S. mutans HARV cultures grown in biofilm-promoting media containing 11 mM glucose and 10 mM sucrose. Although few changes were observed in metabolite production between the microgravity and normal gravity HARV cultures, expression of 154 genes was up-regulated ≥ 2-fold under microgravity growth, and 94 genes were down-regulated ≥ 2fold under microgravity growth. These included a number of genes potentially involved in stress responses. Collectively, these results

suggest that microgravity-induced changes in S. mutans gene expression and physiology may alter the cariogenic potential of this organism during space flight missions.

68. EFFECTS OF BACTERIAL NITRIC OXIDE SYNTHASE ON STAPHYLOCOCCUS AUREUS PHYSIOLOGY

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Staphylococcus aureus is a devastating pathogen, which can infect most tissue and organ systems. Bacterial nitric oxide synthase (NOS) enzymes produce nitric oxide (NO) and have been implicated in survival during in vivo infection as well as resistance to exogenous oxidative stress in several bacteria. However, the contribution of saNOS to S. aureus physiology has not been extensively investigated. Previously published studies have confirmed a role for saNOS in virulence in vivo as well as protection from exogenous oxidative stress. Our data suggests that endogenous reactive oxygen species (ROS) levels are increased in the nos mutant compared to wild-type and complement strains. Furthermore, assessment of cellular respiration with fluorescent stains shows that respiratory activity and membrane potential are increased in the nos mutant, possibly due to increased flow of electrons through the respiratory chain. This increased respiratory activity may therefore be contributing to increased ROS accumulation in the nos mutant. In an attempt to understand how NOS affects the overall physiology of S. aureus, RNAseg analysis was completed on wildtype and nos mutant cultures. Multiple genes associated with oxidative and nitrosative

stress, as well as anaerobic metabolism were altered in the nos mutant. The staphylococcal respiratory response regulator (SrrAB) is a proposed sensor of the reduction state of respiratory quinones. Many of the genes altered in the nos mutant are regulated by SrrAB. Growth curves of a nos srrAB double mutant present with an altered growth phenotype, which can be complemented by nos in trans. A role for NOS in general S. aureus physiology has not previously been established, but our studies presented here suggest a role for saNOS in preventing endogenous oxidative stress and limiting respiratory activity by a currently unknown mechanism. Dissecting the mechanisms by which saNOS regulates these aspects of bacterial physiology could lead to new therapeutic targets against S. aureus infection.

69. MALDI-TOF MASS SPECTROMETRY AND BLAKPC GENE PHYLOGENETIC ANALYSIS OF AN OUTBREAK OF CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE STRAINS.

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Carbapenem-resistant Klebsiella pneumoniae isolates are an important cause of nosocomial infections. In this study a rapid and cost-saving method, based on MALDI-TOF technology, was evaluated and compared with phenotypic, genotypic and epidemiological data for characterization of KPC-Kp strains consecutively isolated during a supposed outbreak.

Twenty-five consecutive KPC Klebsiella pneumoniae isolates were identified and clustered by the MALDI Biotyper (Bruker, Daltonics). To display and rank the variance within a data set Principal Component Analysis (PCA) was performed. The ClinProTools models were generated to investigate the highest sum of recognition capability and cross-validation. A Class dendrogram of isolates was constructed using ClinproTool. MLST was performed sequencing gapA, infB, mdh, pgi, rpoB, phoE and tonB genes. blakpc and cps genes were typed. Phylogenetic analysis and genetic distance of the KPC gene were performed using the MEGA6 software.

PCA analysis defined two clusters, cluster I and II, which were identified in a dendrogram by both temporal split and different antimicrobial susceptibility profiles. These clusters were composed mostly by strains of the same sequence type (ST512), the most prevalent ST in Italy, and the same cps (type 2). In cluster II blakpc genotype resulted more variable than in cluster I. Phylogenetic

analysis confirmed the genetic diversity in both clusters supported by the epidemiological data.

Our study confirms that MALDI-TOF can be a rapid and cost saving method for KPC K.pneumoniae isolates epidemiological clustering and that its association with blakpc genotyping represents a reliable method to recognize possible clonal strains in nosocomial settings.

70. MUCOSAL VACCINATION PRIMES NK CELL-DEPENDENT DEVELOPMENT OF CD8+ T CELLS FOR PROTECTION AGAINST PULMONARY BRUCELLA INFECTION

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Brucellosis is the most common zoonotic disease worldwide, usually transmitted from Brucella-infected livestock after consumption of contaminated foods or by aerosol exposure. Since no vaccines for humans are available, we developed a live, double-mutant Brucella abortus (BADM) strain that after mucosal vaccination, confers complete protection, but abated in IFN- γ -/- mice. Pulmonary infection of naive mice with wild-type BA fails to recruit innate and adaptive lymphocytes to the lungs. Thus, we hypothesize that BADM's protection is tied to innate cell stimulation. To test this hypothesis, 5 days after nasal BADM vaccination, mice showed increased lung IFN- γ producing ILCs and EOMES+ NK cells by 3- and

6-fold, respectively; CD4+ and CD8+ T cells increased 3- and 5fold, respectively. By 2 wks, CD8+ T cells were the dominant IFN-γ source, being 15-fold > naive lungs. After pulmonary challenge, BADM-vaccinated lungs showed fewer CD4+ T cells, but a net increase in CD8+ T cells unlike naive mice, showed no differences in their numbers of CD4+ and CD8+ T cells. To assess if NK cells impact CD8+ T cells, vaccinated mice treated with anti-asialo GM1 Ab to deplete their NK cells, resulted in increased splenic weights, but reduced lung neutrophil influx 5 days after vaccination relative to isotype-treated, vaccinated mice. By 14 days, NK cell depletion reduced by 4-fold (P<0.001) the IFN-γ-producing CD8+ T cells. Thus, NK cell recruitment enhances protection to pulmonary BA in BADM-vaccinated, but not naive mice, which fail to activate NK cells. Supported by NIH Al123244.

71. NOVEL PENA SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) CONTRIBUTE TO CEFTAZIDIME RESISTANCE IN BURKHOLDERIA PSEUDOMALLEI

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Burkholderia pseudomallei is the causative agent of melioidosis, an emerging multifacteted infectious disease. B. pseudomallei infections are refractory to therapy because of the bacterium's intrinsic and acquired antibiotic resistance. Ceftazidime (CAZ), a 3rd generation cephalosporin is effectively used for melioidosis

treatment. Although CAZ resistance is still relatively rare, resistance in response to therapy does occur. The main player in CAZ resistance is chromosomally-encoded Class A PenA β-lactamase. Mutations that cause PenA over-expression or introduce amino acid substitutions in critical regions of the protein cause resistance to CAZ and other β-lactam antibiotics. B. pseudomallei strain Bp1651, isolated in the USA from a patient with a travel history to Australia, is resistant to CAZ and other β-lactam antibiotics including amoxicillinclavulanic acid (AMC), and exhibits increased carbapenem (imipenem, IPM; meropenem, MER) resistance. Here we determined the CAZ resistance factors of strain Bp1651. Sequence analysis showed that this strain had amino acid substitutions in PenA, which included a previously recognized substitution (S72F) leading to AMC resistance and three previously unidentified amino acid substitutions (T147A, D240G, and V261I). Of these, D240G was recently implicated in contacting CAZ in PenA's active site in some PenA mutants. We introduced the D240G allele into strain Bp82 PenA and measured CAZ susceptibility by determining minimal inhibitory concentration (MIC) using broth microdilution. The results showed that Bp82 with PenAD240G showed increased CAZ resistance (MIC = 8-16 µg/ml) when compared to Bp82 (MIC = $2 \mu g/mI$) and Bp82 Δ penA (MIC = 1ug/ml). Resistance to AMC, carbenicillin and MER was unchanged. In addition, we sequenced penA and its promoter region from serial B. pseudomallei isolates derived from 24 melioidosis patients before and after diagnosis of CAZ and/or AMC resistance. Some of the strains contained previously identified mutations, e.g. C69Y and P167S causing CAZ and S72F causing AMC resistance, respectively. A few strains contained new mutations (e.g. I139M, T147A, A172T, and D240G), of which some, e.g. D240G, were implicated in CAZ resistance. In addition, some strains contained a putative promoterup mutation 78 nucleotides upstream of the penA start codon, which is also present in strain Bp1651, and is predicted to contribute to CAZ resistance by PenA over-expression. Knowledge of these mutations

can be used for PCR and/or sequencing based diagnostics of PenAmediated CAZ resistance.

72. NOVEL TETRACYCLINE RESISTANCE MECHANISM IN A BURKHOLDERIA PSEUDOMALLEI CLINICAL ISOLATE

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Burkholderia pseudomallei is a Gram-negative bacterium and the causative agent of melioidosis. Because of significant acquired and intrinsic antibiotic resistance of these bacteria, B. pseudomallei infections are difficult to treat. We are studying resistance to clinically significant antibiotics in patient isolates from diverse geographic origins because understanding resistance will inform means to overcome it. B. pseudomallei MSHR435 is a doxycycline resistant neurological isolate from northern Australia. Efflux mediated by the BpeEF-oprC pump is to date the only known significant tetracycline resistance mechanism in B. pseudomallei. Deletion of this efflux system showed that it is not responsible for the tetracycline resistance of MSHR435. To elucidate the mechanism contributing to resistance in this strain, mutagenesis using the Tn5based transposon T24 was performed to create a random transposon mutant library. This library was screened for mutants exhibiting a doxycycline susceptible phenotype. Replica plating of 5,320 mutants into Luria Broth containing doxycycline revealed two susceptible mutants. Transposon-chromosomal junction DNA sequences were determined to identify T24 insertion sites using nested PCR with genomic DNA templates. The two mutants contained insertions in two distinct loci. One insertion was in amrB, encoding the membrane transporter component of the resistancenodulation-cell-division (RND) AmrAB-OprA efflux pump. The second insertion was in mlaF, encoding the ATP-binding subunit of a cell envelope spanning retrograde phospholipid transport system that was previously implicated in maintaining outer membrane phospholipid homeostasis. Minimum inhibitory concentration (MIC) determinations revealed that when compared to the parental strain MSHR435 the resistance of the amrB::T24 and mlaF::T24 mutants was reduced 16 and 32 fold, respectively. To verify the roles of the two systems in tetracycline resistance, the amrB and mlaF genes are being deleted from MSHR435. Our data provide evidence for a novel tetracycline resistance mechanism afforded by the synergistic interplay between an efflux pump and altered outer membrane permeability affected by changes in its phospholipid content.

73. STRUCTURAL DIVERSITY OF BURKHOLDERIA PSEUDOMALLEI LIPOPOLYSACCHARIDE AFFECTS INNATE IMMUNE SIGNALING IN MURINE MACROPHAGES

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The soil bacterium Burkholderia pseudomallei (Bp) causes melioidosis, a severe tropical disease with a high mortality rate. High mortality rates of this disease are associated with septicemia, which is triggered by the host response to lipopolysaccharide (LPS) components of the Gram-negative outer membrane. Bp LPS is thought to be a weak inducer of the host immune system, allowing the Bp to become a successful intracellular pathogen. In this study, LPS from different serotypes (A, B, B2) and rough type (non-

serotype) of Bp were purified in large quantities and tested for innate immune responses in RAW264.7 mouse macrophage. LPS, besides that of strain 1026b (serotype A) and MSHR840 (serotype B2), was found to strongly induce the inflammatory mediators TNF- α and iNOS in RAW264.7 cells at low concentrations. Innate and adaptive immunity qPCR arrays were used to profile expression patterns of 84 gene targets in response to the different LPS types. Additional qPCR validation confirmed large differences in macrophage response. LPS from a serotype type B strain and rough strain greatly induced the classical type II inflammatory response, a typical response of Gram-negative infections, while the serotypes A and B2 inductions were moderate. The serotype B and rough LPS was also found to greatly induce a type I interferon response usually associated with viral infections, indicating the immunopathogenesis of these strains is different than immunopathogenesis caused by strains similar to 1026b. The accumulation of autophagic vesicles was also increased in macrophages challenged with highly immunogenic Bp LPS. Furthermore, ESI-MS-MS analysis of the lipidA components of the LPS indicated substantial structural differences among different serotypes. These findings add to the evolving knowledge of host-response to bacterial LPS and can be used to better understand sepsis in melioidosis patients.

74. TWO LEGIONELLOSIS OUTBREAKS ASSOCIATED WITH A HYBRID INDEPENDENT-ASSISTED LIVING FACILITY IN FLORIDA HIGHLIGHT THE IMPORTANCE OF HEIGHTENED SURVEILLANCE AND MAINTENANCE OF LEGIONELLA PREVENTION PLANS

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Background: The Florida Department of Health's (DOH) Food and Waterborne Disease Program (FWDP) investigated two legionellosis outbreaks in the same retirement community facility in 2014 and 2015. The facility is regulated by multiple state agencies, including DOH, the Agency for Health Care Administration (AHCA), the Department of Business and Professional Regulation, and the Department of Environmental Protection.

Methods: FWDP conducted an investigation of legionellosis cases associated with the facility in 2014 and made transmission prevention recommendations. Increased surveillance detected another outbreak among facility residents in 2015. In both outbreaks, the FWDP reviewed risk factors, exposure information and performed facility environmental assessments. Water samples collected were analyzed by the DOH Bureau of Public Health Laboratories (BPHL). The facility hired an independent consultant to collect water samples, which were tested at a private laboratory and BPHL.

Results: Four cases were associated with the 2014 outbreak; all required hospitalization and two were independent living (IL) residents. BPHL identified Legionella pneumophila (Lp) in 65.5% (5/8) of water samples collected. Remediation involved multiple thermal disinfection treatments and installation of a continuous secondary disinfection system. An informal Legionella monitoring program was attempted. One month prior to the 2015 outbreak, the disinfection

system was offline due to equipment failure. Water samples indicated Legionella had recolonized the water system and the private company made recommendations to hyperchlorinate the system. The owner declined. In 2015, seven cases were hospitalized and two died; three were IL residents. Water samples analyzed by BPHL were negative for Legionella. However, the private laboratory detected Legionella in 26.7% (20/75) of water samples. Remediation included a 24-hour hyper-chlorination treatment. Both outbreaks were complicated by the lack of medical records for IL residents. The AHCA license does not require hybrid facility nursing staff to maintain medical records on IL residents or track community-acquired pneumonia cases.

Conclusions: Despite remediation efforts after the 2014 outbreak, inadequate management of facility maintenance may have contributed to recolonization of the plumbing system. Hybrid facilities caring for both IL and nursing home residents result in disease monitoring that goes beyond regulation requirements. Facilities should enforce their prevention plan, have increased awareness, and continued public health surveillance of illness clusters potentially associated with the facility. These outbreaks highlight the importance of owner-approved Legionella prevention planning, to include maintenance and water safety management protocols, as well as conducting enhanced surveillance of hybrid care facilities for multiple types of vulnerable populations.

75. A CYCLIC MIMIC OF HIV TAT DIFFERENTIATES SIMILAR TAR RNAS ON THE BASIS OF DISTINCT DYNAMIC BEHAVIORS

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Efforts towards developing RNA-based drug leads have been challenging because of the complexity and dynamic nature of RNA structures as therapeutic targets. The trans-activation response RNA (TAR) and cognate Tat protein of HIV have long been recognized as a promising anti-viral target, and recent works have identified potentially potent inhibitors of the viral RNA-protein interaction. A new class of such inhibitors, conformationally constrained cyclic peptide mimetics of Tat, has been demonstrated to inhibit HIV lifecycle. We have previously probed the complexity and dynamics of TAR RNAs in their free states, as well as conformational shifting by various peptide and small molecule ligands. In this work, we have used ultrafast dynamics approach to probe the interactions between TAR RNAs and one of the representatives of cyclic peptide inhibitors, L22. Our studies demonstrated that cyclic L22 specifically recognize TAR RNAs with at a unique single binding site compared to two binding sites for linear Tat protein. Although both Tat and L22 bind the TAR RNAs as a beta hairpin structure, cyclization in L22 affords it a more efficient ligand from population shifting perspective. This study provided unique insights into drug design with desired properties to target similar structures based on distinct dynamic behaviors.

76. BAYESIAN SPATIO-TEMPORAL MODELING OF MULTI-PATHOGEN INFECTIOUS DISEASES

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Reducing the burden of infectious disease outbreaks in the developing world is an important challenge facing the global community. A crucial step in mitigating infectious disease outbreaks with targeted interventions is identifying the risk factors and understanding the transmissibility of disease while accounting for the spatiotemporal heterogeneity of infections. This research creates a data-driven modeling framework for important infectious diseases and develops statistical methods for identifying significant drivers of pathogen transmission from large biosocial and environmental data. Our framework is flexible and applicable to diseases caused by multiple co-circulating pathogens, such as dengue, cholera, and hand, foot and mouth disease. We apply the method to analyze annual hand, foot and mouth disease outbreaks in China.

77. CO-EVOLUTIONARY ANALYSIS OF PROTEINS FORMING VIROPLASM-LIKE STRUCTURES DURING ROTAVIRAL INFECTION

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Rotavirus replication, with its 11 genes coding for 6 structural (VP1-4, VP6 and VP7) and 5 non-structural (NSP1-5) proteins, constitutes a complex process.

The formation of cytosolic inclusions corresponding to viral machinery of replication responsible for viral template transcription, dsRNA replication and assembly of new viral cores, namely viroplasms, is crucial step in rotavirus replication. Little is known about their formation; however three viral proteins were shown to be important, NSP5, NSP2, and VP2. Nevertheless, the interaction sites of these proteins are far from being definitively elucidated and remain the subject of investigation.

In order to better analyze these interactions, we used several bioinformatic approaches to: (i) analyze possible evidence of recombination events using genetic algorithm for recombination detection, (ii) detect co-evolving residues and to identify significant associations among sites applying a Bayesian graphical models method with a high posterior probability, and (iii) predict in silico the 3D structure of NSP5 to compare the co-evolutionary sites with the structural interactions.

The results showed that the three proteins did not show any significant evidence of recombination. Bayesian network analysis revealed that the co-evolutionary sites are mapped not only to the

deep groove in the monomeric structure of NSP2, which was reported as the interacting site for the other components of viral replication, but also outside this region. The inter-coevolutionary analysis of NSP2-NSP5 showed a clear relationship with sites mapped within the NSP2 groove and the NSP5 region that potentially interacts with it, while other sites are mapped outside these regions. The inter-coevolutionary analysis of NSP2-VP2 showed the involvement of sites within the NSP2 groove, but most sites mapped to outside their supposed regions of their interaction. The analysis of NSP5-VP2 showed several co-evolutionary sites and any interpretation is an assumption in the lack of information about their structural interactions.

The co-evolutionary sites showed evident relationships among these three proteins. Although the groove of NSP2 seems to be the common region for the interaction with NSP5 and VP2, the co-evolutionary sites are also mapped outside the groove region, which suggests that the interactions are governed by several domains to ensure their stability. The prediction of 3D structure of NSP5 showed potential interaction with NSP2, within and outside the groove, which further supports the co-evolutionary analysis performed. Further analysis of the 3D structure of VP2 and its interactions with NSP5 and NSP2 needs to be performed in order to confirm the obtained results.

78. CO-INFECTIONS WITH HEPATITIS C VIRUS AND TUBERCULOSIS AMONG PERSONS LIVING WITH HIV IN AN ONGOING FLORIDA COHORT

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Background: Florida has the highest incidence of new HIV infections in the country, and HIV incidence is increasing each year. In 2014, the racial/ethnic distribution of the 6132 newly-reported HIV infections in Florida was 47% African American, 21% Hispanic, 30% white, and 2% other. Co-infections with hepatitis C virus (HCV) and tuberculosis (TB) continue to be clinical and research priorities for persons living with HIV. This study's objective was to describe the prevalence of HCV and TB among persons enrolled in an ongoing HIV cohort study in Florida, and to compare self-reported diagnoses with medical and laboratory data.

Methods: Recruitment began in October of 2014 and currently includes both urban (Orange, Hillsborough, Broward, and Miami-Dade Counties) and rural (Alachua, Sumter and Columbia Counties) sites. Participants completed a baseline survey that includes items to assess self-reported history of positive HCV or TB test results. Medical chart abstraction is conducted for a majority of cohort

participants, and we are comparing self-reported diagnoses to findings from medical chart abstractions.

Results: 556 persons enrolled in the Florida Cohort from October, 2014- December, 2015. Of these, 63% were African Americans, 11% Hispanic, 25% White, and 1% other. The gender distribution was 59% male, 37% female, and 4% identifying as transgender. 15% reported a history of a positive Tuberculosis test, while 22% of the participants reported a history of HCV diagnosis. Chart abstraction demonstrated some inconsistencies with self-reported HCV diagnosis, but identified at least 12% with confirmed positive HCV antibody, half of whom (6% of the total sample) have laboratory-confirmed untreated HCV as demonstrated by HCV viremia in the previous year. At least 5% of persons also have past or current hepatitis B infection.

Conclusions: Co-infections are common among persons with HIV in the Florida Cohort project, and 90% of these participants have agreed to be contacted about additional studies. We need to identify barriers to treatment for HCV, as new antiviral treatments are available. We are continuing to conduct validation studies to compare self-reported diagnoses to confirmed medical or laboratory diagnoses, and to compare characteristics of those with co-infections to those without co-infections.

79. DROSOPHILA MELANOGASTER SIGMA VIRUS: GENETIC TRENDS IN A MODEL SYSTEM

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Parasite-host interactions are driven by a competition for resources in which the parasite reduces the fitness if its host. This competition provides a visible evolutionary force on host population dynamics. Hosts can counteract these influences through resistance. Resistance changes the control dynamic of parasite-host interactions and can change the persistence of the parasite populations. This effect of resistance on host and parasite populations can be seen in a model system of Drosophila melanogaster and Drosophila sigma virus (DMelSV).

DMelSV is a negative single-strand RNA virus (Rhabdoviridae). When Drosophila melanogaster are infected with DMelSV, they become paralyzed and die when exposed to concentrated carbon dioxide. Progeny from infected females have a longer development time and a lower egg to adult viability. Despite these reproductive costs to the female host, the virus is able to persist in wild Drosophila populations via biparental vertical transmission.

Genetic variation in host resistance can change the coevolutionary dynamics between host and parasite, even potentially driving a parasite extinct. Drosophila melanogaster is known to have genetic variation for resistance to DMelSV. However there have yet to be any systematic attempts to quantify resistance to DMelSV in Drosophila melanogaster. We evaluated 45 unique Drosophila lines for resistance to DMelSV. Flies were artificially infected via injection with sigma virus. Survivors were allowed to reproduce, and multiple generations of their offspring were tested for DMelSV. We evaluated

whether or not all lines were equally vulnerable to infection and found that this was not the case.

80. EFFECT OF THE INFECTION BY A VERTICALLY TRANSMITTED PARASITE ON THE RESPONSE TO ARTIFICIAL SELECTION ON HOST DEVELOPMENT TIME IN THE DROSOPHILA MELANOGASTER/SIGMA-VIRUS SYSTEM.

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Because parasites directly use host resources for their own development and/or activate costly mechanisms of defence, we expect parasites to affect resource allocation between host lifehistory traits. Here we propose to test this assumption by investigating the effect of infection on the response to artificial selection on development time and its correlated effects in the Drosophila melanogaster / Sigma virus system. For 20 generations, three development time treatments, fast, intermediate and slow, were applied to 6 fly lines. Each line was present as either infected or uninfected. Development time assays on uninfected lines from flies of the last generation showed the expected pattern: flies from the fast treatment of selection developed faster than flies from the intermediate and slow treatments of selection. In the infected lines, flies from the intermediate treatment of selection on average had a faster development time than the two other treatments. Virus titer in the infected lines was also found to be the highest in the intermediate treatment of selection. Infection by Sigma virus infection slows down the development time of D. melanogaster. Perhaps as a result, half the infected lines went extinct in the fast development time treatment, suggesting that in the fast treatment, only flies with a low virus titer (and hence relatively fast development time) were selected. The relatively low titer in flies from the last generation could be interpreted as the consequence of

a positive correlation between development time and offspring quality in regards of fighting against parasite infections. Put together, those results suggest the existence of a trade-off in the Drosophila melanogaster – Sigma virus, where the advantages of having a fast generation time, regarding for instance the access to reproduction, could be counterbalanced by a decrease in the ability of fighting again parasite infection. Those results permit a new understanding of the effect of Sigma virus and maybe other arthropod-borne viruses on their host biology.

81. EVALUATION OF ANTIVIRAL ACTIVITY OF RIBAVIRIN AND INTERFERON A 2A AGAINST CHIKUNGUNYA VIRUS

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Introduction: Chikungunya virus (CHIKV) is a mosquito-borne virus that affects millions of people around the world, including the United States. There is currently no antiviral therapy or vaccine licensed for CHIKV, making this virus a significant public health concern. Ribavirin and interferon-α have demonstrated broad spectrum antiviral activity in vitro against a wide range of viruses. Previous studies suggest that ribavirin and interferon are effective against CHIKV as monotherapy and are synergistic when used as combination therapy in vitro. We sought to evaluate the antiviral activities of both these agents as monotherapy and as combination against CHIKV.

Hypothesis: We hypothesized that ribavirin and interferon- α would inhibit CHIKV production in a dose-dependent manner, but at

concentrations that would be considered supratherapeutic in patients due to toxicity.

Method: Vero cells were infected with 181/Clone 25 CHIKV at an MOI 0.0001 in the presence of increasing concentrations of ribavirin and/or interferon- α . Viral production was quantified by plaque assay on vero cells.

Results: Ribavirin inhibited CHIKV in a dose dependent manner on day 1 post infection. However, by day 2 the antiviral effect was largely lost with all experimental arms yielding similar titers. Interferon- α also inhibited CHIKV replication in an exposure-dependent fashion. Combination therapy with ribavirin and interferon- α were synergistic when high concentrations of both compounds were used. At day 2 post infection, RBV EC50 is 2623 µg/ml and INF EC50 89530 IU/ml

Conclusion: Ribavirin and interferon- α display antiviral activity against CHIKV, but high concentrations of both compounds must be used in order for these compounds to be effective in vitro. These findings must be compared to the therapeutic windows for ribavirin and interferon- α to determine if these agents are appropriate for the treatment of CHIKV infection in patients.

82. ISOLATION OF COMMON MIDWIFE TOAD VIRUS AND A RECOMBINANT FROG VIRUS 3 STRAIN FROM NORTH AMERICAN BULLFROGS (RANA CATESBEIANA)

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Ranaviruses are emerging pathogens impacting cultured and wild poikilothermic vertebrates around the globe. Strains of Frog virus 3 (FV3), the type species for the genus Ranavirus, have been implicated in significant epizootics among endangered fish, amphibians, and reptiles. Recently, a distinct and virulent ranavirus strain has been characterized from wild European amphibians (e.g. Common Midwife Toad Virus, CMTV) and cultured Chinese giant salamanders (Chinese Giant Salamander Virus, CGSV). Although the origins of CMTV/CGSV remain obscure, our recent Next Generation Sequencing (NGS) efforts revealed CMTV/CGSV was responsible for an outbreak in North American cultured bullfrogs in 1998. Thus, this outbreak becomes the index case for CMTV/CGSV expanding its host and geographic range. In 2006, the same facility experienced another outbreak in cultured bullfrogs; however, this time a strain of FV3 was isolated. Subsequent experimental infection trials demonstrated that this bullfrog FV3 strain exhibits significantly greater virulence as compared to other FV3 strains across a range of challenged

amphibians. Interestingly, our NGS data suggests that regions of the genome of this bullfrog FV3 strain has recombined with the CMTV/CGSV strain previously isolated from the same ranaculture facility. Our study is the first to demonstrate that bullfrog ranaculture facilities can generate chimeric ranavirus strains of increased virulence. Additionally, the global trade of North American bullfrogs for food may have resulted in the dissemination of a lethal ranavirus strain (i.e. CMTV/CGSV) to naïve European and Asian amphibian populations.

83. MAPPING HIV INTEGRATION SITES USING THE ISLA ASSAY

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In human immunodeficiency virus (HIV) positive patients, HIV infects immune cells and preferentially integrates into introns of transcribed genes of the host cells' genome as a DNA provirus.

Recent studies have provided evidence that genes in cancerassociated pathways 1,2 or that are in close proximity to the nuclear pore3 are more likely to be targets of HIV integration.

While the introduction of combined antiretroviral therapy (cART) has proven effective at eliminating circulating HIV and extending the lifespan of HIV-infected patients, higher incidence of cancer in HIV-infected patients has persisted.

Phylogenetic trees of HIV from infected patients on effective cART demonstrated clades of identical provirus were found in certain tissues, including tumors.

HIV integration site mapping has shown that HIV integration sites are unique and offers the opportunity to confirm if these clades are due the proliferation of identical provirus through clonal expansion of infected cells.

Examining these sites provides more evidence towards understanding HIV persistence in patients undergoing cART, as well as insight into the origins of cancers in HIV-infected patients and the proliferation of HIV in tumors.

Our research group utilized the Integration Site Loop Amplification (ISLA) assay for sequencing integration sites and their corresponding HIV env gp120 sequence.1

Using genomic DNA from a HeLa-derived macrophage cell line that was HIV infected in culture, our efforts produced fifteen total integration sites, during experiments over several weeks.

Five of the integration sites were found at the exact same intronic location in an oncogene, Guanine Nucleotide Exchange Factor (VAV2).

Unique integration sites in other cancer-associated genes, Ribosomal protein S6 kinase beta-2 (RPS6KB2) and Ral Guanine Nucleotide Dissociation Stimulator (RALGDS), were also identified.

Three identical sites were found in the gene ATPase Type 13A3 (ATP13A3). While ATP13A3 is not directly associated with cancer, increased ATP13A3 expression is documented in squamous cell carcinomas.4

Two identical insertion sites were identified in the introns of both Protein Phosphatase 6, Regulatory Subunit 2 (PPP6R2) and Vacuolar Protein Sorting-Associated Protein 53 Homolog (VPS53) genes.

These results confirm the ISLA assay's ability to identify clonally expanding HIV- infected cells.

Understanding the involvement of HIV integration in the clonal expansion of HIV-infected immune cells and cancer pathogenesis provides a more complete understanding of HIV and host dynamics.

This knowledge could influence the development of more effective antiretroviral treatments, as well as methods to prevent oncogenesis in HIV patients, and provide a better quality of life to patients on long-term cART.

84. NEXT GENERATION SEQUENCING TO DIAGNOSE ELUSIVE VIRUSES IN WILDLIFE CLINICAL CASES

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Endangered species under human care may be at a higher risk of papillomavirus infection, especially at breeding facilities where pathogen transmission may be higher. Papillomaviruses are small dsDNA epitheliotropic viruses with a remarkable diversity in highly studied species. They are about 8 Kb in size and contain up to 8 genes, including early genes (E1, E2, E4, E5, E6, E7, and E8) and late genes (L1, & L2). Some of the early genes (E5, E6, E7, and E8) are considered potentially oncogenic. We present two cases of papillomavirus in which molecular diagnosis was not possible using conventional PCR/sequencing diagnostics protocols. The first case occurred in the critically endangered Somali wild ass, Equus africanus somaliensis, with presented typical sarcoid lesions that metastasized over the time. Equine sarcoid is usually caused by Bovine papillomavirus (BPV), which is the only reported case of host switching in papillomavirus. The second case occurred in the flying fox, Pteropus vampyrus. This bat presented a genital wart like lesion that progressed into squamous cell carcinoma. In both cases, clinical lesion and pathology results suggested papillomavirus as the causative agent. However, papillomavirus sequences could not obtained even after several attempts, so we turned to next generation sequencing on the Illumina Miseq to test the preliminary diagnosis. We found a novel papillomavirus in the case of the flying fox, Pteropus vampyrus Papillomavirus -1 (PvPV1), and BPV in the case of the Somali wild ass. Both viruses were almost fully sequenced and with high sequence coverage. Comparison of L1 gene

indicate that this PvPV1 has a 66% amino acid identity to Saimiri sciureus PV1, it closest relative. In the case of the BPV, we can see an unusual possible case of integration in the host genome which could explain the difficulty in diagnosing it via traditional PCR/ sequencing tools. This report stresses the importance of integrating pathology, clinical work, and molecular virology to effectively diagnose and manage pathogens in wildlife species under human care.

85. POTENTIAL RESERVOIRS OF REPLICATING HIV-1 FOUND IN PATIENTS WHO DIED WITH NO DETECTABLE VIRAL LOAD WHILE ON EFFECTIVE DRUG REGIMENS

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Background: Combined anti-retroviral therapy (cART) used to treat HIV-1+ patients is universally effective in reducing plasma HIV load to undetectable levels and restoring partial immunity. However, viral populations rapidly rebound once therapy is removed, and sometimes patients develop drug-resistant HIV while on cART. Furthermore, HIV-associated co-morbidities, particularly certain

cancers, occur at elevated rates even with effective cART. We hypothesized that HIV-infected immune cells in tissues, such as macrophages, may protect HIV from cART leading to on-going HIV replication and evolution.

Methods: The AIDS and Cancer Specimen Resource (ACSR) provided 36 post mortem tissues from five HIV+/cART+ patients with no detectable viral load. All five (designated Pt1-5) died with a form of metastatic cancer (lymphoma, lung, prostate, anal). Tissues were assessed for HIV using droplet digital PCR (ddPCR) and an assay specific for gag, the most conserved HIV gene. Following simultaneous RNA and DNA extraction from the tissues, single genome sequencing was used to generate HIV DNA and RNA sequences for three viral genes: env gp120, nef and pol. Patient-specific sequence alignments were created for the three patients with sufficient sequences from multiple tissues. Maximum-likelihood phylogenies were inferred and multiple statistical tests were performed to investigate viral evolutionary patterns and compartmentalization.

Results: The genomic DNA from all tissues was HIV+ by ddPCR. HIV env gp120 and nef DNA sequences were generated from 21/36 tissues, and five of these HIV+ tissues also contained HIV RNA. Analysis of the estimated proviral copy number between env gp120 and pol sequencing showed a significant difference. Maximum-likelihood env gp120 and nef phylogenies and statistical testing showed little evidence of viral compartmentalization among tissues or between RNA and DNA. These trees contained clades showing evidence of on-going evolution as well as clonal expansion. Testing of pol sequences did not indicate drug resistance mutations were present.

Conclusions: Our results suggest that various tissues may offer a privileged environment for persistent HIV replication during cART.

The discordant numbers of integrated provirus between env and pol sequencing is not surprising, given that env shows more variability. Ongoing viral expression in a subset of tissues in two patients indicates an uneven level of cART penetration in tissues. Two distinct patterns of tissue virus evolution suggest that different modes of replication/spread underlie this persistence: cART-resistant HIV-infected tissue-resident immune cells like macrophages with persistent evolution and migration of HIV infected cells that clonally expand.

86. PREVALENCE OF PSEUDORABIES AT MACARTHUR AGROECOLOGY RESEARCH CENTER

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Pseudorabies (PRV), aka Aujenszky's Disease, is a herpes virus that impacts the health of multiple species. The only natural host is swine and the virus causes neurologic dysfunction and death when infecting other species. The virus is shed through mucous membranes, so oral, nasal, genital, and blood samples were collected from hogs at various sites in Florida. We compared prevalence of pseudorabies antibodies from samples collected at MacArthur Agro-Ecology Research Center at Buck Island Ranch to

other areas in south central Florida and across the state. After DNA extraction, samples were screened for the glycoprotein B gene utilizing qPCR techniques. Of 162 samples collected from 58 animals at MAERC, one blood sample and one oral swab, each from a different individual, tested positive for PRV gB. Prevalence of PRV at MAERC is lower than other areas in the region. Since the virus has been known to recirculate during periods of stress, the decreased prevalence could be due to less human disturbance or increased resource availability than at other sites. Unlike the other sites, MAERC is not open to public access or hunting, activities that could provide a stressful environment for wildlife.

87. PSEUDORABIES (PRV) EXPOSURE AND INFECTION STATUS IN FERAL SWINE POPULATIONS OF FLORIDA

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Feral swine (Sus scrofa) are the most widely distributed invasive wild ungulate in the United States and there are estimated to be > 500,000 individuals in Florida. This species is the disease reservoir for pseudorabies virus (PRV), which is deadly to native wildlife and causes economic losses to the swine industry worldwide. To evaluate

the PRV exposure and infection status in feral swine populations of Florida, we sampled blood, nasal, oral and genital swabs from 522 individuals at 41 public and private sites during 2014-2015. Animals were euthanized as part of population control efforts by USDA/WS/NWDP or collected by hunters. Glycoprotein B enzymelinked immunosorbent assay (gB ELISA) and real-time polymerase chain reaction assay (gB qPCR) were conducted to assess PRV exposure and viral shedding, respectively. Of 411 feral swine tested for PRV exposure, 217 (53%) were PRV-antibody positive, and 37 of 522 (7%) feral swine were viral-DNA positive. Sub-adults had higher PRV infection rates than adult and juvenile feral swine (12% vs. 6% and 3%, respectively), and females had higher viral-DNA prevalence than males (9% vs. 4%). Of 409 feral swine tested for both PRV exposure and infection, 14 (3.4%) animals were PRV-antibody negative and PRV-DNA positive (38% of qPCR positive samples), suggesting that animals actively shedding the virus may be underestimated by only considering PRV seropositivity. Twelve (2.9%) animals were both PRV-antibody and PRV-DNA positive (32%) of qPCR positive samples), suggesting an advanced or stressreactivated viral infection. A relatively high number of animals, 203 (49.6%), exhibited detectable PRV-antibodies, but not viral-DNA, which would indicate that these individuals were either exposed but not infected or had latent infections. Spatial analysis showed that extrinsic factors (hunting, land cover) might influence the persistence and reactivation of PRV, increasing the likelihood of disease transmission among feral swine and other domestic and wildlife species.

88. SHEDDING OF NONINFECTIOUS PARTICLES DURING SPRING VIREMIA OF CARP VIRUS INFECTION: CAN QPCR PREDICT TCID50 OUTCOMES?

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Spring Viremia of Carp Virus (SVCV) is a pathogenic rhabdovirus associated with massive die-offs in both farmed and wild stocks of freshwater fish, plaguing the aquaculture industry worldwide. Quantitative PCR (qPCR) is the current tool of choice for evaluating viral load within host tissues and in environmental samples. During qPCR, the viral genome is targeted and amplified, resulting in the production of many additional copies of the nucleic acids. More nucleic acids in a sample results in faster replication during PCR cycling, and a lower cycle threshold value (Ct; that is inversely proportional to the amount of nucleic acid in the sample). Ct values can be related to the nucleic acid copy number by generating a standard curve from serial dilutions of the qPCR target. Viruses, however, can produce copious noninfectious virus particles with notable quantitative variation between virus species. These products are amplified along with the nucleic acids from the infectious virus, thus contributing to Ct values. Therefore, Ct values may not justly determine how much infectious virus is present in a given sample; i.e., a sample with a relatively low Ct value could actually be free of virus capable of infecting/replicating within host cells. The TCID50 (50% Tissue Culture Infectious Dose) dilution assay is a faithful, gold standard technique used to determine the titer of an infectious virus in cultured cells. TCID50 is achieved at the virus dilution where cytopathic effect (CPE) occurs within only 50% of the cell culture wells. This study aims to establish the relationship between gPCR Ct values and TCID50 outcomes for SVCV. Serial

dilutions used to determine TCID50 values will be amplified by qPCR to discern of the proportion of infectious virus present a sample, and the limit of detection for both assays. Outcomes using this approach will bridge an important gap that has previously interfered with discerning quantitative virus shedding in fish health research studies with SVCV and other viral pathogens.

89. THE ASSOCIATION BETWEEN COLLEGE FOOTBALL GAMES AND GENITAL HERPES DIAGNOSES AMONG UNIVERSITY STUDENTS: AN ANALYSIS OF 9 YEARS OF ELECTRONIC MEDICAL RECORDS

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Binge drinking occurs widely at tailgates and parties during college football games, and such behavior is a known risk factor for sexually transmitted infections. Here, we quantify the association between college football and genital herpes (GH) diagnoses at a Student Health Care Center from 2006-2014 using electronic medical records and publicly available football data.

To account for variable latent periods we subtracted 6 days from the visit date and assigned each visit the most recent Saturday's football game characteristics. We performed zero-inflated compound Poisson regression with sex, age, year and football game characteristics as predictors of daily GH diagnoses.

Controlling for all covariates, the odds of having zero GH cases decreased significantly following in-division, away games (OR=0.39 and 0.36 for lost and won games, respectively), and out-division home games that were won (OR = 0.63) compared to "no game" visits. In-division home games had decreased odds of zero GH cases, though this was not statistically significant (OR=0.28 and 0.60 for lost and won games, respectively). GH cases following won, out-division, away games, and lost, out-division, home games were associated with significantly greater counts (OR=1.67 and 1.39, respectively). Oppositely, GH cases following lost in-division games were associated with lower counts (OR = 0.75 and 0.70 for away and home games, respectively), though this was not significant.

In-division, away games predicting zero GH cases could be explained by fewer tailgates. Out-division games are usually "easy wins" that result in excess celebration. Football games and tailgates may be relevant locations for interventions.

90. THE IMPACT OF RESOURCE ABUNDANCE ON PATHOGEN INVASION RISK

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Territorial animals share a variety of common resources, which can be a major driver of conspecific encounter rates. We investigate how changes in resource density influence the rate of encounters between individuals in a population. We develop a model of resource selection by consumers on a spatial resource landscape and estimate changes in encounter rates as a function of resource availability. Using simulations and asymptotic analysis we show that the relationship between resource availability and consumer encounter rate is nonmontonic. We also find that the maximum distance at which consumers are able to detect resources greatly influences the expected consumer encounter rate. We discuss these theoretical results in the context of a jackal population which has access to a seasonally varying number of carcasses and their subsequent vulnerability to rabies virus outbreaks.

91. TRANSCRIPTOME ANALYSIS OF THE FRONTAL LOBE IN SIV INFECTED MACAQUES TO EXAMINE EFFECTS OF SIV BRAIN INFECTION AND SIV ENCEPHALITIS

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HIV-associated dementia (HAD) is an Alzheimer's-like condition marked by noticeable cognitive impairment and degeneration observed in a subset of patients with human immunodeficiency virus (HIV) infection in the brain. This disease can advance into HIV-encephalitis (HIVE). Insight into the gene expression alterations

induced by HIV brain infection may provide valuable information regarding the pathogenesis of HAD. Rhesus macaques infected with simian immunodeficiency virus (SIV) offer an excellent animal model for the study of the HIV infection and HAD/ HIVE, while avoiding the challenges that arise with studying human HIV brain infection.

This project utilizes the macaque model to study gene expression differences detected between SIV-infected macagues with and without brain infection. These gene expression comparisons aim to identify the biological processes associated with SIV brain infection and SIVE and provide a model for the biological impact of HAND and HIVE. CD8-T-cell depletion prior to SIV infection causes an accelerated disease course, and higher incidence of brain infection. Six non-CD8-depleted (ND) and five CD8-depleted SIV-infected macagues' frontal lobe total RNA samples underwent sequencing with the Illumina NextSeq system at the ICBR. Three of the ND macagues did not develop SIV brain infection. The other three ND macagues developed SIV brain infection; one developed SIVE. The five CD8-depleted macaques all developed SIV brain infection, one of which developed SIVE. The gene expression levels of the samples were compared based upon different disease states, including SIV brain infection (BI), SIVE, and no SIV brain infection (NBI). Paired-end reads were trimmed using Trimmomatic and mapped to an Ensembl reference macaque genome with Tophat utilizing Bowtie2. Differentially expressed genes were identified using Cuffdiff, NOISeqBIO, and Limma. Functional annotation was conducted on significant genes using Gene Ontology.

Several genes enriched in BI and SIVE were involved in pathways such as the Alzheimer disease-presenilin pathway, Huntington disease pathway, and chemokine and cytokine signaling mediated inflammation pathways. Some genes identified as enriched in NBI over BI and SIVE are involved with the Nicontinic acetylcholine receptor-signaling pathway.

Comprehension of which biological pathways are influenced by SIV BI and SIVE could provide molecular markers for HAD diagnosis or possible biological targets for the mitigation of HAD symptoms. Understanding how dementia symptoms arise with HAD may provide insight into the manner of progression of other forms of dementia and brain disorders.

92. TRANSMISSION DYNAMICS OF EBOLA VIRUS DISEASE AND INTERVENTION EFFECTIVENESS IN SIERRA LEONE

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Sierra Leone is the most severely affected country by an unprecedented outbreak of Ebola virus disease (EVD) in West Africa. Although successfully contained, the transmission dynamics of EVD and the impact of interventions in the country remain unclear. We established a database of confirmed and suspected EVD cases from May 2014 to September 2015 in Sierra Leone, and mapped the spatiotemporal distribution of cases at chiefdom level. A Poisson transmission model revealed that the transmissibility at the chiefdom level, estimated as the average number of secondary infections caused by a patient per week, was reduced by 43% (95% CI: 30%, 52%) after October 2014 when the strategic plan of the United Nations Mission for Emergency Ebola Response was initiated,

and by 65% (95% CI: 57%, 71%) after the end of December 2014 when 100% case isolation and 100% safe burials were essentially achieved. Population density, proximity to Ebola treatment centers, cropland coverage, and atmospheric temperature were variables associated with EVD transmission. The household secondary attack rate (SAR) was estimated to be 0.059 (95% CI: 0.050, 0.070) for the overall outbreak period. The household SAR was reduced by 82% from 0.093 to 0.017 after the nationwide campaign to achieve 100% case isolation and safe burials had been conducted. This study provides a complete overview of the transmission dynamics of 2014-2015 EVD outbreak in Sierra Leone at both the chiefdom and household levels. The interventions implemented in Sierra Leone seem effective in containing the epidemic, particularly in interrupting household transmission.

93. AN ONTOLOGICAL ANALYSIS OF SYMBIOTIC RELATIONSHIPS

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Over the better part of a century and a half, collaborative efforts to pin down a standard definition for 'symbiosis' have been mostly unfruitful, with a number of interpretations receiving alternating use in the scientific literature. Two common conflicting definitions are (1) any interspecies interaction in nature and (2) only mutually beneficial interspecies interactions. One consequence of this situation is that the burden of resolving such discrepancies gets passed on to those scientists responsible for constructing scientific

ontologies who may be less familiar with the subject matter and who therefore may adopt an ad hoc approach that only accounts for a fraction of the domains in which this terminology is used. This approach often precludes effective integration with other ontologies since it offers an incomplete representation of reality. We defend the former definition over the latter common interpretation as a more appropriate standard on the grounds that it provides more descriptive power for characterizing the plurality of interspecies interactions found in nature, while also maintaining a greater degree of harmony between the logical structure of biomedical ontologies and the entities represented therein. More specifically, we cast this problem in the context of infectious disease scenarios, doing so for two reasons: (1) we are developing an ontology for epidemic modelling, and (2) the microscopic interactions that occur within this domain mirror and are in some cases more complex than those that occur at more macroscopic levels. Our view rests upon the notion that a less restrictive definition for 'symbiosis' that encompasses all interspecies interactions is preferable both for eliminating inconsistencies with how it and any associated terminology are used, and for constructing formal representations of these interactions and the organism roles associated with them. We believe that, because our methodology focuses on the logical and ontological (i.e., regarding the nature and general features of reality) manner in which interspecies interactions are observed to exist in reality, the formal representations we present here will provide an adequate foundation with which biological scientists and ontologists from various backgrounds can use in their ontologies. Furthermore, we believe that our view may help to augment the progress that has been made toward the realization of a universally-accepted standard definition for 'symbiosis' and its accompanying terminology.

94. ASSESSMENT OF FOOD SAFETY RISKS AT FARMERS' MARKETS IN FLORIDA

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With consumers' growing interest in fresh produce consumption and support of local economy, the number of farmers' markets has significantly increased over the past decade. However, the increasing popularity has also raised food safety concerns for food sold at farmers' markets. The objective of this study was to assess the food safety risks linked to farmers' markets by surveying market conditions and food safety practices at the markets. For this goal, 25 farmers' markets from 10 counties in North and Central Florida were selected based on their schedule and location. Selected markets were visited more than once between April and October of 2014 and observation of current market conditions and vendor practices were made and recorded. Market conditions that were observed included availability of hand washing facility, animal presence, waste management, and separation of items sold at markets. This study showed only 22 % of surveyed markets employed any kind of food station segregation strategies (e.g. food items from non-food items; raw meat from ready-to-eat food). Majority of the markets (64%) had animal presence on the day of survey. Lack of a hand-washing facility was a major problem observed in this study. Of 25 markets surveyed, 72% did not have any bathroom or hand washing facility on site, and 8% of markets relied on portable toilets without any separate hand washing facility. On vendor practices, only 14% of

vendors used gloves while handling food. The results from this study indicate that there is significant lack of food safety practices at farmers' markets, mostly due to limited regulations or guidance on food safety at farmers' markets. Knowledge from this study will help develop effective food safety education programs for vendors, managers and consumers of farmers' markets.

95. BIOFILM EVALUATION ON EXPLANTED ORTHOPEDIC IMPLANTS

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Biofilms are communities of bacteria which behave differently than single-celled organisms. The study of these biofilms is an emerging and diverse area of microbiology spanning both of basic and applied sciences. The identification of how they form, what kinds of microorganisms they are composed of, and their characteristics are widely important to the field of medicine and public health. Biofilms commonly form in wounds or surgical implant sites and can be nonresponsive to treatment with antibiotics or other commons disinfectants. Some progress has been made in treating biofilm infections in chronic skin wounds, but other fields still require more insight. The chronicity and lack of response to antibiotics, suggests that some failed orthopedic implant surgeries may be due to biofilms. Explanted hardware cannot be cut without destroying the biological materials attached to it, so we sought to develop a wholesurface staining technique to identify and localize bacteria on the explanted hardware. For experimentation, explanted porcine skin coupons are used as a model to begin development of the staining method. After fixation with formalin, a chromogenic immunohistochemical staining protocol is performed to stain the entire surface of the skin. The stained surface is then imaging via macrophotography to localize where biofilms have formed. Noninfected pieces of skin are used as negative controls. The ultimate goal is to stain orthopedic hardware removed from patients with suspected infections to determine if the explanted hardware contain biofilms or are pathways to infection (sutures). The results from this study will provide context for future research and will improve knowledge for surgeons and researchers alike. Chronic wounds are an important issue to public health, and biofilms are believed to contribute largely to the difficulty in treating these wounds. We hypothesize that biofilms are also responsible for some failed orthopedic implants. Research into this area may not only advance medicine, but also industries that are impacted by biofilms such as shipping, sewage treatment and microbial fuel cells.

96. COASTAL SEAFOOD CONSUMPTION POST-DWH IS >200 FOLD HIGHER THAN NATIONAL ESTIMATES: OPPORTUNITIES FOR IMPROVED RISK ASSESSMENT IN SEAFOOD SAFETY

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Seafood consumption patterns in Gulf coastal communities were discerned to address public health risks associated with seafood consumption following the Deepwater Horizon oil spill. We developed and implemented a food frequency questionnaire to

analyze seafood consumption and body weights from households of seafood workers, fishers and other residents in Gulf coast communities in Florida and Alabama. Data from over 900 individuals revealed that seafood consumption in Gulf coast communities is higher than national estimates based on 2003-2010 NHANES data (FDA levels of concern for PAH contaminants in seafood are based on NHANES national consumption and body weight estimates). Upper percentile seafood consumption of our study participants were 231 and 298% higher for finfish, and 536% and 984% higher for shellfish (shrimp, crab and oyster), than upper percentile national estimates for adults and youth, respectively. The marked differences between community-specific seafood consumption and NHANES estimates underscore the need to consider local and regional consumption rates when developing risk assessment models. Further, seafood consumption patterns varied substantially between communities based on local heritage, fisheries and economics. These outcomes provide important perspectives that bridge environmental and public health, and underscore the utility of community-based science to define more protective estimates of risk.

97. CORRECTING THE ANALYSIS OF INFECTIOUS DISEASE TRANSMISSION IN HOUSEHOLDS

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Household surveillance studies of infectious disease attempt to estimate the probability of transmission from an infected household member to each susceptible person in the household, which is called the household secondary attack rate (SAR). Many of these studies treat the number of secondary infections in a household of size m as a binomial(m-1, p) random variable, where p is the SAR. This assumes that all infections in the household are caused by the index case. Since the probability of a transmission chain of length k is p^k and p is small, it is thought that the possibility of multiple generations of transmission within the household can be ignored. However, the number of possible transmission chains from the index case to a given susceptible increases with the length of the chain. There are m-2 possible paths of length 2, (m-2)(m-3) paths of length 3, and so on. Because of this, the probability of multiple generations of transmission within the household can be much higher than is normally assumed. Estimates of p that assume a single generation of transmission within the household overestimate the actual SAR and produce confidence intervals with poor coverage probability. To estimate the SAR correctly, a chain-binomial model or contact interval model must be used. Here, we outline these theoretical results and demonstrate them in simulations.

98. DETERMINANTS OF ANIMAL OWNERSHIP IN PERI-URBAN HOUSEHOLDS OF WESTERN KENYA

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Background: Animal husbandry is important to many urban and periurban communities of sub-Saharan Africa. Although it provides an opportunity to complement incomes and increase the available food for household members, urban and peri-urban animal husbandry poses a serious risk for the spread of zoonotic and reverse zoonotic disease between humans and animals. Zoonotic disease can be transmitted through direct contact, the ingestion of contaminated food or water, via vectors, and by inhaling contaminated particles from the air. Domestic animals expose livestock keepers, household members, and communities to public health threats through improper animal, environmental, and household management practices. The study examined geographical, sociocultural, and economic household determinants of animal ownership in three peri-urban 'slums' of Kisumu, Kenya. Through a household survey, participants reported on the types of animals owned, animal care and management practices, the purpose of the animal, and differences in household member contact. Understanding the role of animals in urban and peri-urban households could lead to the development of One Health interventions aimed at creating a healthier relationship between man, animal, and environment in these communities.

Methods: Between February and April 2015, 800 households participated in an extensive cross-sectional survey spanning topics

related to household access to water and sanitation, hygiene practices, animal ownership and contact, gender-based stress and violence, child health and care, and community waste management. Households within the peri-urban slum communities of Nyalenda A, Nyalenda B, and Obunga were selected using a two-stage probability cluster sampling design. Descriptive statistics were employed to describe the households with animal ownership as well as animal types, purpose of ownership, and household member animal contact. Bivariate and multivariate logistic regression analyses using explanatory variables within the categories of geography, sociocultural factors, and household economic were used to investigate potential predictors of animal ownership. Statistical significance was defined by a two-tailed p-value ≤ 0.05.

Results: Of the participating households, 34% (n=252) reported owning at least one kind of animal (livestock, poultry, or companion). Households with animal ownership were primarily male-headed (73%) compared to female (27%). Most households with animal ownership rented the home, land, or both (71%) and the majority of households noted that at least one type of animal sleeps inside the house (n=191). Of household members, adult males were reported to have the most contact with livestock (22%) followed by adult females (20%). However, adult females had more contact with poultry (59%) and companion animals (31%) than adult males (7% for both). Most animals were owned for income, trade, or sale (45%), as a source of meat/eggs (58%), for milk production (31%), and as pets (29%). Male-headed households primarily owned chickens (60%), cattle (46%), and cats (39%). Female-headed households largely owned chickens (56%), cats (44%), cattle (19%) and goats (19%). The multivariate analysis using significant results from the bivariate analysis found several determinants for animal ownership. Ownership of property (coefficient= -0.24) was negatively associated with animal ownership while ownership of

agricultural land (coefficient= 0.11) was positively associated. Social networks within the community were also significant. Perceiving a strong or very strong community bond (coefficient= 0.15) and membership in community groups (coefficient= 0.75) were positively associated with animal ownership.

Conclusion: Household animal ownership characteristics and contact in the peri-urban slums of Kisumu, Kenya vary by factors related to geography, sociocultural norms, and economics. However, this study shows the importance of property and land ownership as well as community involvement in explaining whether a household owns animals. Community groups may represent a platform for communicating One Health messages on animal husbandry safety.

99. EBOLA EPIDEMIC: USING CURRENT EVENTS TO TEACH AUTHENTIC INQUIRY SCIENCE

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First reported in March of 2014, the Ebola Virus Disease (EVD) outbreak in West Africa has now claimed more lives than all other known Ebola outbreaks combined, making it the deadliest occurrence of the disease since it was first discovered nearly 40 years ago. In the hopes of turning the outbreak into something positive from an educational standpoint a module was developed focusing on EVD, infectious disease, and epidemiology. The module described in this paper engages students in a series of inquiry-based lessons, providing accurate and up-to-date information on the current outbreak of Ebola in West Africa. The lessons also serve to correct popular misconceptions about the disease. The lessons include: a jigsaw activity in the form of a webquest using resources from the Centers for Disease Control, a simulation based on fluid exchange to model the spread of an outbreak of infectious disease,

and a "disease detective"-style mapping activity based on published data from the New England Journal of Medicine outlining the start of the current Ebola outbreak in Guinea.

100. EFFECT OF ADAR MUTANTS ON DROSOPHILA MELANOGASTER FITNESS AND THE INTERACTION WITH DMELSV (SIGMA VIRUS; RHABDOVIRIDAE)

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Activity of the ADAR gene in Drosophila melanogaster is largely deleterious in flies, although it plays a role in neuronal function. However, an antiviral activity of ADAR has been proposed. This study aims to determine if there is an effect on virus replication and hence host fitness due to ADAR, using the vertically transmitted virus DMeISV. The ADAR gene codes for an enzyme that edits doublestranded RNA. ADAR alters the nucleotide sequence by deaminating Adenosine (A) causing it to become Inosine (I). The A to I change ultimately results in A being replaced by Guanosine. DMelSV reduces host fitness by lengthening development time and lowering both female fecundity and hatching success. This study examines the interaction between DMelSV and ADAR on fly viability. The viability of various ADAR loss of function and wild-type flies were compared, both with and without DMelSV infection. Preliminary analysis suggests no significant effect on viability between the infected and the uninfected condition across the different range of ADAR mutations.

101. ENVIRONMENTAL DYNAMICS AND VIBRIO IN AN ECUADOREAN ESTUARINE SYSTEM

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Washington Cardenas; Mark Polhemus

The emergence of waterborne diseases such as cholera, whose causative agent is pathogenic strains of Vibrio, is strongly linked to the local environmental and ecological context. Machala is a port city of 250,000 people in El Oro province, on the southern coast of Ecuador, near the Peruvian border. The 1991-2004 cholera pandemic emerged in Peru and spread north into El Oro, making it a key sentinel site for understanding dynamics in the ongoing 7th pandemic. In Machala, many peoples' livelihoods depend on the estuarine system, from fishing for subsistence and trade, to domestic water use, making the coupled human-estuarine system an important component of public health management. We sampled five estuarine locations twice weekly over a 10-month span, within a gradient of human use, and over a geographic range from inland to ocean, to measure water-specific environmental variables such as pH, temperature, salinity, conductance, and algal concentration, and conducted PCR testing for Vibrio spp., including pathogenic strains, across 5 months. Our sites exhibited considerable seasonal and spatial heterogeneity in environmental variables, with clear peaks during specific months. We found evidence of an environmental reservoir for Vibrio spp., including pandemic strains O1 and O139, but did not confirm ongoing toxigenic presence. We found that the

timing of positive PCR results was coupled to the environment. This study was conducted in a moderately normal climate year, providing a preliminary framework for monitoring coupled Vibrio – estuarine dynamics for potential emergence of cholera outbreaks in the region.

102. HIV/AIDS AND AWARENESS IN THE 21ST CENTURY

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With advent of ART, anti-retroviral therapy, AIDS is no longer a death sentence. When treated effectively, HIV-positive individuals can live long lives and, for many, the terror of an HIV diagnosis has eased since the AIDS epidemic of the 1980s. However, for some, fear remains and infection continues to spread, particularly in Florida. According to the CDC HIV Surveillance Report from 2013, four Florida cities—Miami, Jacksonville, Orlando, Tampa/St. Petersburg—are among the 25 U.S. cities with the highest rates of HIV infection, even as the rate of infection decreases in the South overall.(1) In a CDC review of the most distinctive causes of death by state, Florida led the nation in deaths resulting from HIV.(2) Lack of knowledge is a major contributor to why HIV/AIDS remains a significant health concern. In fact, all aspects of the disease—transmission,

survivorship, quality of life—are impacted by the current state of knowledge among the general public. Through projects made possible by grant funding from the National Library of Medicine, the Health Science Center Library is working to address the knowledge gaps that can prevent individuals from accessing care. Our faculty are collaborating with UF and community partners to provide HIV/AIDS information to health care consumers, clinicians, students, and social service/information providers. Outreach projects include hosting a National Library of Medicine exhibit on Surviving and Thriving and facilitating partnerships with a number of organizations in the community and on campus. This poster will describe the HIV/AIDS problem that affects Florida and particular minority populations in the state disproportionately, the information resources that are available online for both consumers and healthcare professionals, and the projects, programs and groups that are being brought to bear in addressing this problem.

103. IMPACT NETWORK ANALYSIS OF POTATO SEED SYSTEMS IN TUNGURAHUA, ECUADOR

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The potato seed system—a multilayer network—includes social, economic, and agroecological components (e.g., market prices, disease outbreaks, IPM, and seed production). Seed and ware potato

producers, breeding institutions, technical support, and pests and diseases are components of this complex system that interact and modify each other continuously. An "impact network" is the linked socioeconomic and biophysical networks through which new technologies can impact system outcomes. An "Impact Network Analysis" (INA) is an evaluation of how impact networks function in a particular setting such as seed systems, and where the control points for improvement are. We apply the novel concept of INA to understand interconnectivity and impacts on the potato seed system of the Consortium of Small Potato Producers (CONPAPA) in Tungurahua, Ecuador, evaluating the implications of the CONPAPA structure for pest and disease sampling, IPM, and farmer decision making. Identification of key nodes and control points that influence the success of seed systems (e.g., farmers, farms, information, and seed sources) supports enhancement of the system (e.g., maximizing the distribution of new seed varieties using fewer distribution channels, managing disease outbreaks, and targeting improvement of communication and infrastructure). Resources can be invested in particular nodes to improve practices and to control pest and disease outbreaks, leading to improvements in the seed system. We present results for CONPAPA Tungurahua along with general recommendations for improving seed system structure.

104. INVASION AND SATURATION RISK OF PATHOGENS AND PESTS BASED ON GLOBAL CROPLAND CONNECTIVITY

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The geographic connectedness of croplands plays a major role as a risk factor for the invasion of crop-specific pathogens, and the risk of saturation by endemic pathogens. Understanding these network structures supports sampling and management strategies. We evaluated global networks of five vegetatively-propagated crops (banana/plantain, cassava, potato, sweetpotato, and yam) that are key to food security. Diseases transmitted through vegetative propagation are notoriously damaging. We analyzed the structure of the global crop networks, where the potential link between geographic location pairs was evaluated using a gravity model, as a function of the distance between the pair of locations and the product of the harvested crop area in the two locations. Networks were evaluated using a novel index of pathogen or pest invasion and saturation risk, based on the role of locations in bridging cropland areas and the degree of connectedness of a location and its neighbors. For example, in addition to high risk locations with high cropping density, locations with high risk because of their role as bridges for cassava include South-Central Nigeria, Central Ghana,

and Southwestern Democratic Republic of Congo. For potato, bridges include Central and Southern Poland and Northern Ukraine. The highly-linked hub and bridge locations we identified are likely priorities for surveillance and management, and for tracing intraregion movement of diseases.

105. INVESTIGATIONS ON THE COST OF RESISTANCE IN COEVOLVING POPULATIONS OF E.COLI AND T3 PHAGE

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We used Escherichia coli and T3 phage to study the coevolutionary process between host and virus. Microbes are ideal organisms for such research because they have short generational times and large population sizes, and hence evolve quickly. When infecting E. coli, T3 phage targets lipopolysaccharide receptors on the bacteria membrane. This receptor is also involved in important functions for the host fitness, such as the structural integrity of the membrane. Therefore, we hypothesized that gaining resistance to the phage would result in important fitness drawbacks for the bacteria, i.e. that the bacteria would incur a "cost of resistance." We carried two independent communities, A and B, of clonal populations of bacteria and phage in an environment supplemented with glucose for 17 days, and aliquots were saved daily at -80C. We hypothesized that the coevolution dynamic among communities carried in the same nutritional environment would be similar. Infection assays of community B revealed that the bacteria evolved resistance to the phage gradually; day 1 bacteria showed no resistance, middle time point bacteria showed partial resistance, and day 17 shows an alternate mechanism of complete resistance. The growth rate

experiments for bacteria from days 1, 8, and 17 showed that resistant strains of bacteria presented the greatest fitness costs, with slower growth rates and lower growth plateaus. Infection assays of community A showed a very different coevolution dynamic: bacteria from days 1, 11, and 17 were resistant to day 1 phage, but phage from later days, 4-17, were able to infect and kill the bacteria from all time points. Therefore, phage from population A evolved more counter-adaptations to host defenses than phage from population B. The experiment enhanced our understanding of the mechanisms of host-parasite coevolution, which include the fitness costs that arise when the host evolves resistance to the phage and the variation in coevolution dynamics among independent communities carried in the same nutritional environment.

106. MICROBICIDAL HYDROCOLLOID WOUND DRESSING

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Hydrocolloid dressings are useful to promote wound healing, but they frequently need to release a biocide such as silver ion to limit infections. However, almost all microbicidal agents that are released from wound dressings can impair wound healing. We describe a new dressing that integrates an antimicrobial based on low levels of hydrogen peroxide to provide durable antimicrobial protection for the product while preserving the excellent wound healing characteristics of a hydrocolloid dressing.

This product has recently been cleared for sale by FDA, and is based on a pressure sensitive adhesive formulation of hydrocolloid with a history of safe and effective use in wound healing and in ostomy applications, combined with a superabsorbent that contains this hydrogen peroxide source. The combination forms a hydrocolloid useful for both ostomy and wound healing of low to moderately exudative wounds, including pressure ulcers, and diabetic ulcers.

This formulation kills >99.99% of common wound pathogens including MRSA, Acinetobacter baumannii and E. coli which should help to protect vulnerable patient populations and caregivers as well as to help prevent transmission of pathogens at dressing changes. The hydrocolloids were tested in a porcine model of full and partial thickness skin wounds to assess effects on the rate of wound healing as compared to control materials that included nonstick pads, conventional hydrocolloids and a silver dressing. The hydrocolloid products (both with and without peroxide) demonstrated significant reductions in wound area, especially in days 7 through 14 for full thickness wounds (faster healing), compared to the non-adherent dressing and the silver based controls. In addition, the peroxide containing hydrocolloid dressing showed less apparent scar tissue compared to silver dressing. In summary, this new hydrocolloid wound dressing that incorporates zinc peroxide nanoparticles has broad microbicidal effects across a spectrum of the most serious wound pathogens, and unlike most other microbicidal wound dressings, actually accelerates healing of full thickness skin wounds and reduces scarring compared to typical silver containing wound dressings.

107. MICROBIOLOGICAL SAFETY OF BAKED GOODS SOLD AT FARMERS' MARKETS IN FLORIDA

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Farmers' markets have been gaining popularity in the United States. While fresh produce is the major food item sold at farmers' markets, increasing number of homemade food products are sold at farmers' markets due to the passage of Cottage Food law in many states. And yet there is limited research data on microbiological safety of food sold at farmers' markets, and few studies done on this topic. The goal of this study was to assess the microbiological safety of baked goods sold at farmers' markets in Florida. For this goal, 17 markets in North and Central Florida were selected, and a total of 130 baked goods samples were collected from these markets from Apr to Oct of 2014. During sampling, market conditions and vendor practices were observed and recorded as well to determine the effect of food safety practices on microbiological safety. All samples were prepared based on AOAC method, and tested for the presence of total coliform, generic E. coli, and Staphylococcus aureus. Of 130 samples, one sample was confirmed positive for Staph. aureus by both selective plating and PCR. Also, 0.8% (n=1) and 18% (n= 24) were positive for generic E. coli and total coliform, respectively. While there was no correlation found between total coliform occurrence rate and market locations or market sizes, this study showed that samples handled with bare hands by vendors were 6 times more likely to be contaminated with coliforms than those handled with

gloves on. Additionally, samples from markets with animal presence have 1.6 times higher chance to be positive for total coliforms. The results from this study suggest some of the foods sold at farmers' markets might have been prepared or handled under unsanitary conditions, and food safety can be improved by educating vendors about best food safety practices.

108. RHIZOBIOME RESPONSES TO NEW TOMATO ROOTSTOCK SYSTEMS

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Grafted plants are becoming more popular with tomato growers, due to increased yield and lower incidence of soilborne diseases. The success of grafted tomato plants could be in part a function of the rhizosphere microbiome, as microbes are critical for plant health and performance. We analyzed the impacts of grafting and rootstock genotypes on bacterial and fungal communities in the roots and the rhizosphere of tomatoes using high-throughput sequencing and network modeling. Our study evaluated rootstocks representing non-graft (BHN589), self-graft, and two hybrid grafts: (BHN_1028 and RST-04-106), while the scion (BHN589) remained the same across the treatments. The experiment was repeated at three study sites at Kansas. Diversity measures calculated based on OTUs revealed significantly (p<0.001) more diverse bacterial and fungal communities in the rhizosphere than in the roots across all the

treatments, although a large proportion of OTUs were shared (85% in bacteria, and 40% in fungi). Evaluation of OTUs unique to each treatment showed microbial taxa specific to rootstock genotypes in both the roots and the rhizosphere. The number of unique OTUs in the rhizosphere was higher than in roots (20 times for bacterial OTUs, and 3 times for fungal OTUs). Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Planctomycetes were the most abundant phyla in all rootstock-scion combinations, where the proportion of the last three phyla was lower in the roots. Pezizales, Cantharellales, and Xylariales were enriched as root endophytes, whereas Pleosporales, Mortierellales, and Hypocreales were the dominant fungal orders in the rhizosphere. Our study shows an effect of grafting and rootstock genotype on the composition of microbial community in both the roots and the rhizosphere. Ultimately, a better understanding of microbial responses to rootstock management will support improved vegetable production, and inform efforts towards phytobiome-based crop breeding and disease management systems.

109. STI KNOWLEDGE IN COLLEGE STUDENTS: IDENTIFYING TARGETS FOR SAFER SEX EDUCATION.

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A growing concern in public health is the incidence of sexually transmitted infections (STIs) among young adults and adolescents. In 2008, and estimated 50% of new cases of STIs in the United States occurred in individuals aged 15 to 24. Although sexual health knowledge is not directly associated with safer sex behaviors, knowledge is crucial to making informed decisions when choosing to have sex. To determine demographic differences in STI knowledge

accuracy, we analyzed data from a cross-sectional survey administered to a random sample of 991 college students aged 18-24 years old. We employed non-parametric statistics to identify differences among gender, race, sexual orientation, STI and HIV testing history, and being sexually active in the past year. In addition, multivariate linear regression was used to determine which of the above variables best explained STI knowledge accuracy. Findings can be used to inform health education practitioners of specific demographic and behavioral groups that can be targeted for sexual health awareness campaigns.

110. THE EFFECT OF ENVIRONMENTAL CHANGE ON THE CHEMOSENSORY ABILITIES OF THE CARIBBEAN SPINY LOBSTER PANULIRUS ARGUS

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The Caribbean spiny lobster supports the single most valuable fishery in Florida and other important fisheries throughout the greater Caribbean. The IPCC 2013 identified the coastal marine ecosystems that these lobsters inhabit as high risk for impacts from anthropogenic climate change. Anthropogenic induced climate change has strong consequences for marine ecosystems, including sea surface temperature rise, sea level rise (SLR), and ocean acidification.

Chemical cues are important at almost every level of marine ecosystems and dictate a wide variety of fundamental biological processes and behaviors. One potential consequence of these

changes is alteration in the chemosensory abilities of marine animals such as the spiny lobster Panulirus argus. Lobsters rely heavily on chemical cues for many biological and ecological activities. Chemical cues from conspecifics and cohabitants are used by spiny lobsters to identify suitable shelter when returning from foraging and cues from predators and diseased individuals are used to determine shelters that are unsafe and need to be avoided. The objective of this study was to determine the effect of some of these environmental changes (temperature, salinity, and acidification) on the chemosensory abilities of P. argus. Animals collected from hard bottom habitat in the Florida Keys were placed in the center of a rectangular raceway and presented with two shelters, one at each end of the raceway. Lobsters behavior to known aggregation and avoidance cues under different environmental treatments were recorded (high salinity, low salinity, high temperature, acidic conditions, and ambient salinity and temperature).

111. VALIDATING APPROACHES TO DETECTING HIDDEN SOCIAL GROUPS

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In the early stages of many infectious disease outbreaks, infected individuals are only minimally symptomatic, but they will often engage in some kind of behavior that makes them unusual (e.g. a sudden increase in clinic visits) relative to the larger population in which they live. Similarly, small terrorist groups will be indistinguishable from a surrounding large urban population except for occasional unusual -- and thus potentially detectable -- behavior. In both instances, rapid detection of the special small groups is important to preventing catastrophic events. The problem of detecting a small, essentially hidden or covert group embedded in a

background of "clutter" poses special methodological challenges. Here we use a large empirical dataset concerning human activity -utilization of the municipal wifi system in the city of Montreal over a period of five years -- as an example of clutter. Covert groups, in particular, tend to make themselves as indistinguishable from the background population as possible. They only become detectable, in principle, if there is some facet of their behavior that is unusual, possible only over a short period of time. Here we demonstrate the creation of a synthetic covert group whose wifi system utilization is consistent with that of one or more subgroups of the actual Montreal population. We show that synthetic group behavior can be tuned to match different kinds of interactions and different group organizations. Then with different scenarios of potentially distinguishable behavior by the synthetic group, we assess the ability of several detection algorithms to identify these groups when they are embedded in the real population. A further objective of this overall study is to characterize the combinations of unusual behavior by the synthetic group and detection algorithms that do, and do not, allow for successful identification.

112. WOMEN OF URBAN SLUMS OF KENYA LIVE IN COMPLEX AND STRESSFUL WATER, SANITATION AND HYGIENE CONDITIONS

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Global studies show relations of poor and inadequate WASH conditions in households and communities, and psycho-social stress among women. Thirty one percent of urban Kenyans have access to improved sanitation and 82% have access to improved drinking water sources. Our study aims to understand the relationships of household WASH conditions, and various other determinants of psycho-social stress among women in urban slums of Kisumu, Kenya. Data was collected from three urban sub-locations of Kisumu municipal area of Nyanza province in western Kenya. A mixed methods approach using qualitative and survey data has been used. Semi-structured interviews were conducted with 20 women to understand the socio-economic and living conditions of individual participant household's water, sanitation, hygiene conditions and behaviors, existing challenges, and support mechanisms adopted by the women. Two-stage probability cluster sampling design was used to select the 800 households for a cross-sectional survey. We used qualitative data analysis and structural equation model to describe and test our study aim. A variable on self-reported psychosocial stress has been created by using responses to the Hopkins Stress Syndrome Checklist 10, to measure symptoms of stress among the women. Our calculated stress score has a mean of 1.83, which is higher than our set cut-off score for distress of 1.75 used in other

studies. We found household WASH conditions (Coefficient: -0.02), female decision making (Coefficient: -0.03) and social support (Coefficient: -0.03), to be inversely statistically significant to psychosocial stress. Food insecurity (Coefficient: 0.20) was found to be positively significantly associated to psycho-social stress. Household WASH conditions (Coefficients: -0.05-0.09; -0.02-0.01) is negatively statistically significant with all WASH based violence variables: feeling unsafe to fetch water, feeling unsafe to use toilet, attacked/threatened while using toilet, attacked/threatened while fetching water respectively. The complex relationships of household WASH conditions and threats from poor WASH conditions, women's ability to articulate decisions and seek support to improve her living conditions, household wealth, and food insecurity, influenced by being located in a particular geographical and administrative setting, having adequate education and employment, women's age, and being the head of a household have direct or indirect impact on her being in stress. Complicated and traditional social norms continually restrict women's involvement in decisions regarding sanitation improvement within their houses and in communities. Their lack of participation in sanitation programs acts as a barrier in creating enabling sanitation facilities for them and in gender mainstreaming sanitation and hygiene improvement programs.

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