Tufts University
Research Days 2009 – 2010
Global Health and Infectious Disease Poster Presentations

Monday, October 5, 2009
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Title:
Tracking Cholera Outbreaks from Satellites: Space-Time Variability of Chlorophyll in Northern Bay of Bengal

Authors:
Antarpreet Jutla, Ali Akanda, Shafiqul Islam

Presented by:
Antarpreet Jutla

Department:
Department of Civil and Environmental Engineering, Tufts University School of Engineering

Abstract:
Cholera bacteria exhibit strong association with coastal plankton. Characterization of space-time variability of chlorophyll, a surrogate for plankton abundance, in Northern Bay of Bengal (BoB) is an essential first step to develop any methodology for tracking cholera in the Bengal Delta from space. This study quantifies the space-time distribution of chlorophyll in BoB region using ten years of satellite data. Variability of chlorophyll at daily scale, irrespective of spatial averaging, resembles white noise. At a monthly scale, chlorophyll shows distinct annual seasonality and chlorophyll values are significantly higher close to the coast than in the offshore regions. At pixel level (9 km) on monthly scale, on the other hand, chlorophyll does not exhibit much persistence in time. With increased spatial averaging, temporal persistence of monthly chlorophyll increases and lag one autocorrelation stabilizes around 0.60 for 1200 km2 or larger areal averages. Spatial analyses of chlorophyll suggest that coastal region in BoB have a stable sill at 100 km. Offshore regions, on the other hand, do not show a stable sill. This study puts a lower limit on space-time averaging of satellite measured plankton at 1200 km2-monthly scale to track cholera outbreaks from space in Northern BoB.
Title:
Climate Extremes and Infectious Diseases: Large Scale Hydroclimatic Controls in Forecasting Cholera Epidemics

Authors:
Ali Akanda, Antarpreet Jutla, Shafiqul Islam

Presented by:
Ali Akanda

Department:
Department of Civil and Environmental Engineering, Tufts University School of Engineering

Abstract:
Despite ravaging the continents through 7 global pandemics in past centuries, the seasonal and interannual variability of cholera outbreaks remain a mystery. Previous studies have focused on the role of various climatic and environmental factors, but provided little or no predictive capability. Recent findings suggest a more prominent role of large scale hydroclimatic extremes – droughts and floods – and attempt to explain the seasonality and the unique dual cholera peaks in the Bengal Delta region of South Asia. In Bangladesh, these extreme hydrologic events are typically followed by water-borne disease outbreaks and have tremendous impact on public health. The government and other aid agencies are often caught unaware in such public health emergencies due to the vast nature of the outbreaks. We investigate the seasonal and interannual nature of cholera epidemiology in three geographically distinct locations within the region to identify larger scale hydroclimatic controls that can set the ecological and environmental ‘stage’ for outbreaks. Here we show that 2 distinct, pre and post monsoon, cholera transmission mechanisms related to large scale hydroclimatic controls prevail in the region. An implication of our findings is that extreme events such as prolonged droughts, record floods, and major cyclones may cause major disruption in the ecosystem and trigger massive epidemics. We postulate that a quantitative understanding of these large-scale and dominant processes with significant system memory will form the basis for forecasting epidemic outbreaks. A multivariate regression method using these predictor variables to develop probabilistic forecasts of cholera outbreaks is explored. Forecasts from such a system with a seasonal lead-time is expected to have significant impact on early cholera detection and prevention efforts in endemic regions.
Title:
Protection of Neonatal Mice from *Vibrio cholerae* Infection by Immunization with Outer Membrane Vesicles

Authors:
Anne Bishop, Abdullah Tariq, Bharathi Patimalla, Stefan Schild, Stephen Calderwood, Firdausi Qadri, Andrew Camilli

Presented by:
Anne Bishop

Departments:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine; Howard Hughes Medical Institute; Laboratory Sciences Division, International Centre for Diarrhoreal Disease Research, Bangladesh, India; Institute of Molecular Biosciences, University of Graz, Austria; Division of Infectious Diseases, Massachusetts General Hospital

Abstract:
Not available.
Title:
Silk Biomaterials for Enhanced Stability and Controlled Release of Antibiotics

Authors:
Eleanor Pritchard, Thomas Valentin, Fiorenzo G. Omenetto, David Kaplan

Presented by:
Eleanor Pritchard

Department:
Department of Biomedical Engineering, Tufts University School of Engineering

Abstract:
Typical systemic delivery of antibiotics can be limited by difficulty of penetrating epithelial barrier shells around infections and the risk of liver damage with large doses. In addition, antibiotic instability at temperatures ≥25°C makes transport and storage difficult (particularly in third world clinical settings where refrigeration is limited). A simple system for antibiotic delivery is needed that also stabilizes the incorporated drug, biodegrades to avoid surgical retrieval, and restricts delivery to a specific target site to minimize side-effects and maximize efficacy of dose. This need demands a novel approach to the storage and delivery of antibiotics. Silk, a naturally derived protein polymeric biomaterial, is biocompatible, safe, FDA approved and degrades in vivo to nontoxic products. Further, the unique protein chemistry composition of silk has proven useful for the stabilization of antibiotics as well as other drugs. Antibiotic-loaded silk biomaterials (including films, sponges, gels and microspheres) could be stored for extended periods at room temperature then injected, applied directly to wound sites, or the antibiotic liberated via protease treatment. These materials could deliver antibiotics locally (avoiding systemic side effects) and then degrade naturally over time.

Release of antibiotics from silk films, gels, microspheres and combinations was examined using a bacterial lawn (Staphylococcus aureus, Gram Positive, and Escherichia coli, Gram Negative bacteria) based on zone of inhibition. Long term stability was assessed to compare penicillin stored in solution versus stored in silk films at 4°C (refrigeration), 25°C (room temperature) and 37°C (body temperature). Hydrogels loaded with penicillin or ampicillin sustained release for 48 hours and 4 days, respectively. Penicillin and ampicillin loaded silk microspheres suspended in silk gels released a lower daily rate than bulk loaded gels but continued to release for 4-5 days. Penicillin stability declined rapidly when stored in solution, but penicillin stored in silk films retained activity above 60% for 19 days at all storage temperatures. Over 80% of the original penicillin activity was detected after 12 days of storage in silk films at 37°C (compared to less than 5% for penicillin in solution).

Silk biomaterials are capable of sustained antibiotic release and could be used to deliver both large initial clearance doses and slower sustained maintenance doses depending on mode of processing. Incorporation of
penicillin into silk films substantially enhances stability compared to storage in solution, preserving significant activity even when stored at room temperature and body temperature. We conclude that antibiotics (including penicillin) can be stored in silk biomaterials (at temperatures as high as 37°C) and the drug can be effectively released with bioactivity. These silk-based antibiotic storage and delivery systems would be especially useful in third world clinical settings due to the elimination of the need for refrigeration, ease of transport and storage, and the ability to efficiently release antibiotics locally over sustained timeframes without constant reapplication, multiple repeated injections or a need for surgical retrieval.
Title: Multidisciplinary Team Working Toward Zero Orthopedic Surgical Infections

Authors: Maureen Spencer, Susan Davidson, Diane Gulczynski, Susan Cohen, Stephen Parazin, John Richmond

Presented by: Maureen Spencer

Departments: Infection Control Unit, Department of Infectious Disease, Department of Patient Care Services, Department of Orthopedics and Rehabilitative Services, New England Baptist Hospital

Abstract:

Introduction: A multidisciplinary team was formed to address an increase in the infection rate in FY03 and implement control measures in an orthopedic specialty hospital.

Objective:
- Administration established intent for zero tolerance for surgical site infections.
- The formation of a multidisciplinary task force was established to identify problems and implement corrective action plans and infection prevention measures.

Methods: The team included representatives from OR nursing, orthopedic surgeons, anesthesia and managers from infection control health care quality, central supply, facilities and environmental services. The team evaluated operating room procedures, practices and facility design and prioritized action plans to institute infection control measures. In addition, throughout the 5 year period, reinforcement of hand hygiene was done with creative and highly visible marketing campaigns for staff and visitors.

The specific Issues evaluated each year included.
- 2003 - traffic control, surgical attire, surgical hand scrub, environmental disinfection, processing of surgical instruments, HEPA filtration and laminar flow
- 2004 - antibiotic surgical prophylaxis and silver impregnated post-op dressings
- 2005 - use of antibacterial sutures
- 2006 - prescreening program for MRSA and Staph aureus and decolonization protocol
- 2007 - elimination of the use of locally administered steroids in laminectomy surgery
- 2008 - chlorhexidine skin prep and antimicrobial dressings
Results: Orthopedic infections and rates during the 5 year period were as follows:

- FY03 - 63 SSI/8837 cases (0.7)
- FY04 - 60 SSI/9669 cases (0.6)
- FY05 - 49 SSI/9216 cases (0.5)
- FY06 - 46 SSI/8986 cases (0.5)
- FY07 - 39 SSI/9027 cases (0.4)
- FY08 - 37 SSI/8884 cases (0.4)

Standardized infection ratios were calculated each year by risk index and benchmarked against CDC/NNIS data. These guided the team in risk analysis of the orthopedic population. A 60% decline in MRSA and *Staph aureus* infections was observed after the implementation of the MRSA and *Staph aureus* eradication program. Laminectomy infection rates decreased from 1.3% to 0.5% after discontinuing the routine use of local steroids.

Conclusion: The importance of a team approach to infection control in the operating room is key to a successful infection control and prevention program. Integrating infection control into surgical services is an effective way to foster communication, collaborative work and achieve lower infection rates. We have documented a steady decline in SSI over a five year period of diligent attention to risk analysis and implementation of effective prevention measures.
Title:
Eradication of Methicillin Sensitive *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus*
Before Orthopaedic Surgery

Authors:
Maureen Spencer, Susan Davidson, Diane Gulczynski, Susan Cohen, Stephen Parazin, John Richmond

Presented by:
Maureen Spencer

Departments:
Infection Control Unit, Department of Infectious Disease, Department of Patient Care Services, Department of Orthopedics and Rehabilitative Services, New England Baptist Hospital

Abstract:
Introduction: Asymptomatic colonization with Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA) has been described in the literature as a risk factor for orthopaedic surgical site infection (SSI). Identifying *Staphylococcus aureus* colonization in the pre-surgical screening process is important in reducing SSI. We examined the effectiveness of preoperative surveillance and eradication on colonization rates in orthopaedic SSI.

Methods: We performed preoperative anterior nares surveillance screens of 7,019 patients admitted for orthopaedic surgery using polymerase chain reaction (PCR) assay. The treatment intervention for MRSA and *Staph aureus* patients was a 5-day application of intranasal mupirocin 2% applied twice daily. MRSA positive patients were instructed to bathe with chlorhexidine for 5 days and were rescreened prior to surgery. Contact precautions were implemented if the second screen was positive for MRSA. All MRSA positives received Vancomycin for surgical prophylaxis.

Results: From July 17, 2006 through March 31, 2009, 16,486 patients were screened; 3,957 (23%) were *Staph aureus* positive and 710 (4%) were MRSA positive. Repeat nasal screens were obtained from MRSA patients prior to surgery and revealed 77% eradication. In the cohort of positive screens, there were 7/3,957 *Staph aureus* infections (0.18%) and 9/710 MRSA infections (1.28%). In the 11,819 negative screens there were 11 (0.09%) infections (3 MRSA and 8 MSSA). Overall, there were 27 SSI in 16,458 screened patients (0.16%). In an equivalent group of historical controls during the prior year, there were 24 MRSA/MSSA infections in 5,283 cases (0.45%).

Conclusions: We have documented a significant reduction in orthopaedic SSI with the implementation of a MSSA/MRSA surveillance and eradication program for all inpatient surgeries during the preoperative screening process.
Title:
Spectral Cytopathology of Cervical Samples: Detecting Cellular Abnormalities in Cytologically Normal Cells

Authors:
Erin Morris, Jennifer Schubert, Benjamin Bird, Kostas Papamarkakis, Miloš Miljković, Kristi Bedrossian,
Nora Laver, Stephen Naber, Max Diem

Presented by:
Erin Morris

Departments:
Department of Pathology, Tufts Medical Center; Department of Chemistry and Chemical Biology,
Northeastern University

Abstract:
Aim: Spectral Cytopathology (SCP) is capable of detecting intrinsic molecular changes within individual exfoliated cells that are the result of cervical disease caused by human papillomavirus (HPV) infection. SCP is a novel application of Fourier Transformed Infrared Micro-spectroscopy for the objective and unsupervised diagnosis of unstained cells by detecting spectral patterns unique to disease.

Method: Infrared spectral maps of 16 mm² are collected from cells deposited onto low-e slides (Kevley Technologies, OH), which are glass microscope slides coated with a thin layer of Ag/SnO₂. Each cell has a corresponding infrared spectrum and spectral data is preprocessed (2nd derivatives and vector normalization) and is analyzed by unsupervised chemometric algorithms principal component analysis (PCA). This new methodology classifies cells according to their unique cytoplasmic protein content.

Results: In this study, 12 samples of mature squamous cells with normal morphology were scrutinized by SCP; their original cytological diagnoses were as follows: 7 normal samples and 5 samples with low-grade squamous intraepithelial lesions (LSIL). All of the samples came from patients who were currently taking oral contraceptives in order to control hormonal fluctuations. Approximately 200 – 500 cells from each sample were analyzed. SCP accurately diagnosed 10 of the samples according to their cytological diagnosis. The spectral patterns which differentiated these samples are most likely the result of an inherent HPV infection since the remaining 2 samples, although classified as abnormal by SCP, had normal conventional cytological diagnosis and did not show abnormal cell morphology upon re-review of the cells. Furthermore, follow-up investigation revealed that these samples came from patients who had a history of abnormal cytology. It is likely that these cells, which by conventional cytology do not show abnormal cells, are still affected by HPV. The morphologically abnormal cells and morphologically normal cells from 2 confirmed LSIL samples were analyzed by SCP. All cells had similar spectral patterns; the morphologically abnormal cells were not differentiated from the morphologically normal cells.
**Conclusion:** SCP tracks consistent chemical variations in cells which are being transformed by disease states. Therefore, SCP does not depend on identifying the few morphologically abnormal cells within a large sample in order to make an accurate diagnosis, as traditional cytology requires. These results indicate that the biochemical analysis provided by SCP is a more sensitive diagnostic tool for HPV screening in gynecological cytology and can potentially be used in prevention of disease.
**Title:**
Therapeutics Which Inhibit Botulinum Toxin Function and Accelerate Its Degradation Within Neurons

**Authors:**
Chueh-Ling Kuo, George Oyler, Jorge Sepulvedal, Saul Tzipori, Charles Shoemaker

**Presented by:**
Chueh-Ling Kuo

**Departments:**
Department of Biomedical Sciences, Cummings School of Veterinary Medicine; Synaptic Research

**Abstract:**
Botulinum neurotoxin (BoNT) is the most potent bacterial toxin, causing disease and creating a serious Category A bioterror threat. Seven BoNT serotypes A-G inhibit neurotransmission by delivery of a protease to neurons that cleave proteins necessary for exocytosis. BoNT/A is the most persistent and dangerous serotype, with symptoms lasting up to a year in humans. In this project, we seek to accelerate intraneuronal BoNT protease degradation by exploiting the host proteasome ubiquitin system. A series of constructions were prepared and tested that are composed of a BoNT protease targeting domain and an E3 ligase/E3 ligase targeting domain. These ubiquitin E3-ligases, once delivered to intoxicated neurons, should bind the BoNT protease and target it for accelerated ubiquitination and turnover. The BoNT protease targeting domains are camelid heavy-chain antibody fragments (VHH). Some of the VHHs are also potent inhibitors of the protease activity. Among all of the E3 ligase targeting domains tested, we found that the F box protein, TrCP, was most effective. To date, we developed E3 ligases consisting of an anti-BoNT/A protease domain fused to the TrCP F-box domain. These ligases substantially accelerate BoNT/A protease degradation in cell based assay and protect neuronal cells from the consequences of BoNT/A intoxication. Replacement of the BoNT protease targeting domain a VHH specific to BoNT serotype B protease resulted in an E3 ligase promoting BoNT/B protease degradation. We are currently developing a means to deliver the designer ligases to the cytosol of intoxicated neurons by fusing them to atoxic mutants of Clostridial toxins. If successful, it should be possible to develop therapeutic E3 ligases against all serotypes by selecting appropriate BoNT protease targeting fragments.
Title:
The Role of International Veterinary Medicine in Global Health and Infectious Disease Prevention and Control

Authors:
Robyn Alders, Joann Lindenmayer, Gretchen Kaufman, George Saperstein, Eric Brum, Stacie Lawson

Presented by:
George Saperstein

Department:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine

Abstract:
The International Veterinary Medicine (IVM) Program at Tufts University Cummings School of Veterinary Medicine is a nationally and internationally recognized leader in the field of international veterinary education, and in innovative approaches to supporting animal health research in the developing world.

The IVM Program is a key component of the overall Tufts University international focus, working closely with multiple organizations in sustainable livelihood support, disease prevention and control, and public health. Internationally, the IVM program works in partnership with a variety of organizations, including the International Livestock Research Institute, the Food and Agriculture Organization of the United Nations (FAO), Nepal’s National Veterinary School, the Institute of Agriculture and Animal Science (IAAS) of Nepal’s National Tribhuvan University, Nepal’s National Zoonoses Centre, the University of Gadjah Mada in Indonesia, KYEEMA Foundation in Southern Africa, the University of Minnesota, Development Alternatives Inc., the United States Agency for International Development (USAID) and the United States Department of Agriculture’s Agricultural Research Service. The program is currently collaborating with the USAID Avian and Pandemic Influenza and Zoonotic Disease Program and with the FAO in Indonesia on the implementation of their Highly Pathogenic Avian Influenza Control Program.

Since the first Cummings veterinary student went to Niger in 1982, over 250 students have taken advantage of international research opportunities during their time at Tufts. This experience has enabled a significant number of students to move successfully into international veterinary careers after graduation.
Title: Traditional Water Resource Management as a Determinant of Human Health in Rural Tanzania: Examining Water Quality and Quantity

Author: Ayron Strauch

Presented by: Ayron Strauch

Department: Department of Biology, Tufts University School of Arts and Sciences

Abstract: Traditional resource management (TRM) is the application of customs and local ecological knowledge (LEK) to regulate the utilization of resources. TRM often provides an effective alternative to western principles of resource management, especially when natural resources are variable and unpredictable. Many examples of TRM and LEK have focused on the utilization of plant, animal or marine resources, but few have detailed the impacts of TRM on water resources. Water resources are vital in rural East African communities where quality is important for drinking and quantity is important for personal, domestic and environmental hygiene. Water-related diseases are particularly common in the region, the seasonality of which is generally linked to climatic patterns. After examining water resources and water use behavior I describe the water quality benefits of TRM in rural Tanzania by comparing water resources from traditionally managed catchment forests to conventionally managed catchment forests. Bacterial data and qualitative data gathered in the field substantiates the claim that water from traditionally managed forests is of higher quality and preferred by villagers to water from conventionally managed forests. I conclude that TRM can provide additional benefits for communities that are not provided by other management strategies, including unrealized benefits in public health through reductions in water-related diseases.
Title:
Evaluation of an Educational Campaign to Increase Hand Hygiene at a Veterinary Teaching Hospital

Authors:
Annie Shea and Scott Shaw

Presented by:
Annie Shea and Scott Shaw

Department:
Foster Hospital for Small Animals, Cummings School of Veterinary Medicine

Abstract:
Objective: To establish baseline data on rates of hand hygiene behavior and to evaluate the effectiveness of an educational intervention aimed at improving hand hygiene.

Design: Observations before and after an educational intervention.

Sample Population: Veterinary medical faculty members, students and technicians at a small animal teaching hospital.

Procedures: Proper hand hygiene was defined as use of anti-bacterial foam or hand-washing before or after physical interactions between providers and patients. Data were collected by anonymous direct observation. After an initial observation period, a multi-modal educational campaign promoted proper hand hygiene with specific attention to increasing use of anti-bacterial hand foam. Rates of hand hygiene post-intervention were collected to evaluate the effectiveness of the program.

Results: At baseline, 20% (117/568) of observed interactions met criteria for proper hand hygiene; after intervention, 42% (78/187) (p<0.01) of observed interactions met criteria. Use of antibacterial foam increased from 6% to 36% (p<0.01). An unconditional logistical regression analysis showed that people were 4.1 (p<0.01) times more likely to wash or foam after the intervention than before after adjusting for ward, position and shift.

Conclusions: Initial low rates of proper hand hygiene at baseline were improved substantially and maintained two months after implementing a low cost multi-modal educational campaign.
Title:
Using a Model with Two Types of CTL Regulation and Two Types of Infected Cells to Understand Virus Dynamics in SIV Vaccination Trials

Authors:
Rebecca Batorsky, Rinat Sergeev, Igor Rouzine

Presented by:
Rebecca Batorsky

Departments:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine; Loffe Physical Technical Institute, St. Petersburg, Russia

Abstract:
Understanding the failure of vaccination trials requires detailed analysis of interaction between HIV/SIV and the immune response. In vaccinated animals, the virus is not cleared, but still establishes a persistent state, although the average virus load is lower than in unvaccinated animals by 1.5 decimal logs. Yet, the effect of vaccination is much smaller than for SHIV vaccination trials.

Previously, we developed a rather detailed mathematical model based on a range of dynamic features of HIV and SIV infection, including CTLs responding directly to infected cells, and CTLs responding to CD4 helper cells activated by small amounts of virus. The model predicted two stable steady states, one state with a high virus load and few helper cells (>104 RNA/ml), as in a typical untreated individual, and a state with a very low virus load and a high number of helper cells (<10 RNA/ml), as in the long-term non-progressors. In fact, vaccinated monkeys and many unvaccinated individuals appear to be in a third steady state with intermediate properties: High frequencies of CTLs and helper cells but intermediate virus loads.

To interpret the third-state properties, we considered several modifications of the model. One includes, in addition to short-lived highly-productive infected cells, chronically infected cells with low levels of virus production and weak immunogenicity. Numeric simulation with four fitting parameters (other model parameters were fixed) provides a close fit of viremia dynamics observed for vaccinated rhesus macaques from Norman Letvin’s lab. The model predicts that chronically infected cells dominate the steady-state viremia. Few short-lived infected cells are responsible for maintenance of constant helper cell response and helper-dependent CTLs. To test our explanation, future experiments need to focus on relative contributions to the virus load from infected cell compartments with different virus production rates.
Title:
Curriculum Co-Development in Public and Ecosystem/Environmental Health: An Innovative E-Learning Method

Authors:

Presented by:
Jeffrey Griffiths

Departments:
Departments of Public Health and Community Medicine and of Anatomy and Cellular Biology, Tufts University School of Medicine; Technology for Learning in the Health Sciences, Tufts University; Department of Environmental and Population Health, Cummings School of Veterinary Medicine; Department of Civil and Environmental Engineering, Tufts University School of Engineering; Makerere University, Kampala, Uganda; Muhimbili University of Health Allied Sciences, Tanzania; Kenya Methodist University, Kenya; Department of Veterinary Anatomy and Physiology, University of Nairobi, Kenya

Abstract:
African universities have historically endured dwindling resources, outdated libraries, and limited access to current information and global discourse. This has been accentuated by the digital divide between North and South, and has contributed to the loss of professionals (“brain drain”). Improving ICT provides an avenue for addressing these resource limitations. We describe our experience using a novel educational method, curriculum co-development (CCD), which bridges this gap. CCD is a powerful pedagogical model for higher education. It was developed in the 1990s by African and U.S. faculty, including Professor Pearl Robinson of Tufts University, to collaboratively teach political science. We adapted CCD to health education and deliver novel interdisciplinary curricula in public and ecosystem health between Tufts University and a university consortium in East Africa. Constraints are related to slow and costly internet connections. These will improve with new undersea cables to East Africa.

CCD starts with face to face faculty meetings to compare and agree on topics and curricular materials to be shared. The teaching materials are then hosted on the internet and made available to participating faculty and
students. Depending on ICT robustness, synchronous and asynchronous teaching and tutored mentoring on discussion boards is conducted by the involved faculty. Medical, veterinary, dental, public health, nutrition, pharmacy, nursing, and engineering faculty and students have engaged in our shared teaching. We use the Tufts University Sciences Knowledgebase (TUSK) e-learning platform, a knowledge management system which includes digital health sciences libraries and mobile telephone connectivity. CCD has improved the curriculum at each institution, provides global perspective, and respects the insights and wisdom of each site. It enables institutions in regions with the greatest global health challenges to be fully represented in finding appropriate solutions.

This innovation bypasses educational resource limitations, linking students and educators across institutional and international boundaries in a community of truly global health education and practice.
Title:
A Proof of Concept Thermostable Measles Vaccine

Authors:
Jeffrey Mariner, Luke Ascolillo, Sue Lautze, Jeffrey Griffiths

Presented by:
Jeffrey Griffiths

Departments:
Department of Public Health and Community Medicine, Tufts University School of Medicine; International Livestock Research Institute, Nairobi, Kenya; University of Oxford, Oxford, United Kingdom

Abstract:
An heat-stable measles vaccine is urgently needed, given cold-chain misadventures and 500,000 measles deaths yearly. Measles virus descended from rinderpest virus and is physiochemically nearly identical. We therefore hypothesized that methods used to develop the thermostable rinderpest vaccine (TRV) at Tufts University and the USDA would also apply to measles. TRV is thermostable for >9 months at 37°C, and its widespread use underpins the current successful rinderpest eradication campaign. Edmonston strain measles vaccine virus was grown in Vero cells. Aliquots from a single crude batch of vaccine virus were stabilized with 5% lactalbumin hydrolysate and 10% sucrose, frozen under varying conditions and then lyophilized per Mariner et al. 1990. Vials of lyophilized vaccine were then incubated at 37°C and periodically titrated in cell culture. The thermostability at 37°C of batches frozen at -20, -40, -80, or in liquid nitrogen was 33, 88, 110, and 122 days respectively, to a threshold of 1000 tissue-culture-infective-doses. This measles vaccine preparation is more thermostable than any other yet reported in the refereed literature, while using widely accepted, inexpensive excipients for stabilization. We believe an optimized measles preparation will demonstrate very extended thermostability and low cost, similar to TRV.
Title: Air Pollution and Anemia as Risk Factors for Pneumonia in Ecuadorian Children

Authors: Aaron Harris, Fernando Sempértegui, Bertha Estrella, Ximena Narváez, Juan Egas, Mark Woodin, John Durant, Elena Naumova, Jeffrey Griffiths

Presented by: Jeffrey Griffiths

Departments: Department of Public Health and Community Medicine, Tufts University School of Medicine; Corporación Ecuatoriana de Biotecnología, Quito, Ecuador; Central University of Ecuador, Quito, Ecuador; Department of Civil and Environmental Engineering, Tufts University School of Engineering; Gerald J. and Dorthy R. Friedman School of Nutrition Science and Policy; Department of Biomedical Sciences, Cummings School of Veterinary Medicine

Abstract:

Background: Ambient air pollution and malnutrition, particularly anemia, are risk factors for pneumonia, a leading cause of death in children under five. We simultaneously assessed these risk factors and their interaction in Quito, Ecuador.

Methods: We identified two socioeconomically similar study sites in Quito with higher and lower exposures to particulate matter (PM2.5) and nitrogen dioxide. We enrolled 408 and 413 children aged 18 – 42 months in each neighborhood, and obtained medical histories of physician visits or hospitalizations during the previous year, anthropometric nutrition data, hemoglobin and blood oxygen saturation, and, in a sub-sample, urine tests for the polycyclic aromatic hydrocarbon 1-hydroxypyrene.

Results: Children with higher air pollution exposure and anemia had an increased risk for prior pneumonia hospitalizations (OR=6.82, 1.45 – 32.00; P=0.015), compared to children without anemia (OR 1.04, NS). Overall, higher pollution exposure children had significantly more pneumonia hospitalizations (OR=3.68, 1.09 – 12.44; P=0.036), total respiratory illness (OR=2.93, 95% CI 1.92 – 4.47; P<0.001), stunting (OR=1.88, 1.36 – 2.60; P<0.001) and anemia (OR=1.45, 1.09 – 1.93; P=0.013) compared to lower air pollution exposure children. They also had very significantly lower blood oxygen saturation (92.2% + 2.6% vs. 95.8% + 2.2%; P<0.001), consistent with air pollution related carboxyhemoglobinemia. Urinary 1-hydroxypyrene did not vary by neighborhood.

Conclusions: Ambient air pollution and anemia appear to synergistically increase pneumonia hospitalizations. Improving nutrition-related anemia, as well as diminishing air pollution, may decrease pneumonia incidence.
Title:
Disentangling Nutritional Factors and Household Characteristics Related to Child Stunting and Maternal Overweight in Guatemala

Authors:
Jounghee Lee, Robert Houser, Aviva Must, Odilia Bermudez

Presented by:
Jounghee Lee

Departments:
Institute of Clinical Research and Health Policy Studies, Tufts Medical Center; Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy; Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
Although the familial coexistence of child stunting and maternal overweight has recently emerged in developing countries, studies that examine child, maternal, household characteristics associated with this dual burden are lacking. The aim of this study was to disentangle nutritional factors and households characteristics associated with child stunting and maternal overweight using multivariable logistic regression. We used data collected in the 2000 Living Standards Measurement Study, a nationally representative Guatemalan sample to select 2,261 pairs with a child aged 12 to 60 months and a mother aged 18 to 49 years. In accordance with the WHO criteria, 18.2% of households were classified as having a stunted (height-for-age Z-score < -2) child and an overweight (body mass index ≥ 25) mother (SCOM) in the study sample. The prevalence of other child and mother pairs was: 33.8% for a stunted child and a normal weight mother (SCNM); 25.2% for a non-stunted child and an overweight mother (NCOM); and 22.8% for a non-stunted child and a normal weight mother (NCNM). Socioeconomic status of NCNM pairs was much higher than SCOM and SCNM pairs, but similar to NCOM pairs. Compared with NCNM pairs, SCOM pairs were more likely to have maternal short stature (adjusted OR=3.12, 95% CI=2.08 to 4.69), higher parity (adjusted OR=1.20, 95% CI=1.09 to 1.31), mothers working (adjusted OR=1.67, 95% CI=1.08 to 2.57), and maternal indigenousness (adjusted OR=1.98, 95% CI=1.25 to 3.12). The findings from this study provide new insights to aid tailored nutrition intervention programs that target vulnerable households with a stunted child, an overweight mother, or both.
Title:
Development of an Assay to Detect Polyclonal Antibodies Against Cryptosporidium Mucins in Human Serum

Authors:
Olivia Lai, Christopher Morris, Honorine Ward, Roberta O’Connor

Presented by:
Olivia Lai

Department:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
The parasite Cryptosporidium causes cryptosporidiosis, a diarrheal illness. Cryptosporidiosis can be severe in malnourished children and in immunocompromised individuals. Cryptosporidium hominis and Cryptosporidium parvum are the etiologic agents of human cryptosporidiosis. Both species express the mucins Muc4 and Muc5, polymorphic O-glycosylated glycoproteins. Both mucins are thought to be involved in parasite attachment to, and invasion of the host cell. The immediate goal of these studies was to develop an assay to detect antibodies against Muc4 and Muc5 in the sera of people who had experienced cryptosporidiosis. A cohort of 42 sera was first screened by ELISA for antibodies against gp15, an immunodominant antigen of C. parvum and C. hominis. Sera that were found positive by ELISA were subsequently tested by Western blot for antibodies against native Cryptosporidium antigens to confirm reactivity. Genes encoding Muc4 and Muc5 were amplified by polymerase chain reaction (PCR). PCR products were purified and cloned into the pET46XaLIC vector. The resulting plasmids were introduced in the BL21 strain of E. coli. His-tagged fusion proteins were expressed by induction with IPTG, and purified by metal affinity chromatography. Our next step is to set up the final format of the ELISA for detection of antibodies against Muc4 and Muc5. With this assay in hand, we will determine whether sera obtained from people infected with one species of Cryptosporidium recognizes mucins from the other species.
Title:
Systemic and Mucosal Antibody Responses to *Cryptosporidium* Mucin Antigens in HIV+ Adults in Kenya

Authors:
Patrick Burns, Jane Wanyiri, Honorine Ward, Roberta O’Connor

Presented by:
Patrick Burns

Department:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
*Cryptosporidium* are ubiquitous waterborne parasites that are responsible for outbreaks of diarrheal disease worldwide. They are opportunistic pathogens that cause chronic and sometime fatal diarrheal disease in the immunocompromised. There is currently no effective treatment or vaccine. *Cryptosporidium* uses mucin-like glycoprotein antigens, such as Muc4 and Muc5, to attach to and invade host intestinal epithelial cells. These antigens are highly polymorphic between *C. parvum* and *C. hominis*, the two main species that cause human cryptosporidiosis. Cryptosporidiosis is a major opportunistic infection in AIDS patients in Kenya. Studies of HIV-infected adults in Nairobi showed that 11–17% of HIV-infected individuals with chronic diarrhea had Cp detected in their stool by microscopy. Another study reported that 41% of the *Cryptosporidium*-infected patients died within 4 months and detection of Cp oocysts was the single most significant indicator of death. By measuring serum IgG, IgM and fecal IgA levels to recombinant *C. parvum* and *C. hominis* Muc4 and Muc5 by ELISA in HIV+ patients with symptomatic and asymptomatic cryptosporidiosis, we will be able to determine if systemic and mucosal antibody responses to these proteins are isolate specific and associated with protection from the symptoms. Of the 51 samples collected to date, 9 have been positively identified for asymptomatic cryptosporidiosis by PCR amplification of gp40/15. Preliminary analysis of the Muc4 gene in these samples indicates that the sequence is identical to the published *C. hominis* allele. In contrast, all Kenyan Muc5 sequences were identical to a unique Muc5 allele originally identified in a single Bangladesh sample from a previous study. Results from this study will inform future studies on immune responses to specific antigens in HIV-infected patients and facilitate identification of the correlates of protective immunity.
Title:
The Polymorphic Mucin Antigens CpMuc4 and CpMuc5 are Integral to Host-Parasite Interactions of Cryptosporidium parvum

Authors:
Roberta O’Connor, Patrick Burns, Tin Ha-Ngoc, Kristin Scarpata, Honorine Ward

Presented by:
Roberta O’Connor

Department:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
Cryptosporidium, a waterborne enteric parasite, is a frequent cause of diarrheal disease outbreaks worldwide. Thus far, the few antigens shown to be important for attachment and invasion of Cryptosporidium into the host cell are all mucin-like glycoproteins. In order to investigate other antigens that could be important for Cryptosporidium host-parasite interactions, the Cryptosporidium genome databases were mined for other mucin-like genes. A single locus of seven small mucin sequences was identified on chromosome 2 (CpMucs1-7). RT-PCR analysis demonstrated that all seven CpMucs were expressed throughout intracellular development. CpMuc4 and 5 were selected for further investigation because of the significant sequence divergence between C. parvum and C. hominis alleles. Rabbit anti-CpMuc5 and CpMuc4 antibodies identify several polypeptides in C. parvum lysates, suggestive of proteolytic processing. All polypeptides were larger than the predicted molecular weight indicative of post-translational processing, most likely O-glycosylation. In IFA analysis, both anti-CpMuc4 and 5 antibodies react with the apical region of sporozoites, and identify surface exposed epitopes. The antigens are not shed during excystation, but do partition into the aqueous phase of TX-114 extractions. Consistent with a role in attachment and invasion, CpMuc4 and 5 can be detected binding to fixed Caco2A cells, and anti-CpMuc4 peptide antibodies inhibit Cryptosporidium infection in vitro. Sequencing of CpMuc4 and CpMuc5 from C. hominis clinical isolates identified several polymorphic alleles. The data suggest that these antigens are integral for Cryptosporidium host-parasite interactions and may be potential vaccine candidates.
Title:
The Immune Response to the Hypervariable Domain of Cryptosporidium Glycoprotein gp40

Authors:
Adam Baghban, Roberta O’Connor, Honorine Ward

Presented by:
Adam Baghban

Department:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
Cryptosporidium is a genus of protozoan intestinal parasites which are prevalent worldwide. Cryptosporidium parvum and Cryptosporidium hominis are the species which most commonly infect humans. In immunocompetent hosts, Cryptosporidium infection is often asymptomatic or self limiting. In contrast, Cryptosporidium infection in immunocompromised patients such as those with AIDS, and in malnourished children, may result in serious and life-threatening diarrheal disease. There is currently no vaccine for Cryptosporidium and treatment options are limited. The Cryptosporidium glycoprotein gp40 is important in the interaction between the parasite and host epithelial cells. However, there are extensive polymorphisms in the amino acid sequence of gp40 among Cryptosporidium parvum and Cryptosporidium hominis isolates, possibly because of selective immune pressure. In order to develop an assay to study the antibody response to the hypervariable domains of gp40 from both species, primers specific to this domain from C. hominis subtype IA and C. parvum subtype II were designed. Following PCR amplification from genomic DNA, these sequences were cloned into the pET 46 vector and expressed in E. coli BL21. Recombinant proteins were purified and used as antigen in ELISA to detect antibody responses to gp40 in sera from Bangladeshi children who had acute cryptosporidiosis. Initial results of such experiments showed that the antibody response to the hypervariable domain of gp40 may occur across 2 species and subtypes. However, further experiments are necessary to confirm these findings.
Title:
Molecular Cloning and Characterization of p30, a Galactose/N-acetylgalactosamine-Specific Lectin Which Mediates Cryptosporidium Infection in vitro

Authors:
Najma Bhat, Angela Joe, Mercio Perrin, Honorine Ward

Presented by:
Najma Bhat

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Department of Pathology, Tufts University School of Medicine

Abstract:
Cryptosporidium spp. cause human and animal diarrheal disease worldwide. The molecular mechanisms underlying Cryptosporidium attachment to, and invasion of, host cells are poorly understood. Previously we described a surface-associated Gal/GalNAc-specific lectin activity in sporozoites of C. parvum. Here, we describe p30, a 30 kDa Gal/GalNAc-specific lectin isolated from C. parvum and C. hominis sporozoites by Gal-affinity chromatography. P30 is encoded by a single copy gene containing a 906 bp open reading frame, the deduced amino acid sequence of which predicts a 302 amino acid, 31.8 KDa protein. The p30 gene is expressed during C. parvum infection of intestinal epithelial cells in vitro. Antisera to recombinant p30 expressed in E. coli react with a ~30 kDa protein in C. parvum and C. hominis. P30 is localized to the apical region of sporozoites and is predominantly intracellular in both sporozoites and intracellular stages of the parasite. P30 associates with gp900 and gp40, Gal/GalNAc-containing mucin-like glycoproteins which are also implicated in mediating infection. Native and recombinant p30 bind to Caco-2A cells in a dose-dependent, saturable and Gal-inhibitable manner. Recombinant p30 inhibits C. parvum attachment to and infection of Caco-2A cells while antisera to the recombinant protein also inhibit infection. Taken together, these findings suggest that p30 mediates C. parvum infection in vitro and raise the possibility that this protein may serve as a target for intervention.
Title:
Role of CpSUB1, a Subtilisin-Like Protease in *Cryptosporidium parvum* Infection *in vitro*

Authors:
Jane Wanyiri, Patsharaporn Techasintana, Roberta O’Connor, Michael Blackman, Kami Kim, Honorine Ward

Presented by:
Jane Wanyiri

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Division of Parasitology, The National Institute for Medical Research, London, United Kingdom; Departments of Medicine and of Microbiology and Immunology, Albert Einstein College of Medicine

Abstract:
The apicomplexan parasite *Cryptosporidium* is a significant cause of diarrheal disease worldwide. Previously we reported that a *C. parvum* subtilisin-like serine protease activity with furin-type specificity cleaves gp40/15, a glycoprotein that is proteolytically processed into gp40 and gp15, which are implicated in mediating infection of host cells. Neither the enzyme(s) responsible for the protease activity in *C. parvum* lysates nor those that process gp40/15 are known. There are no furin or other proprotein convertase genes in the *C. parvum* genome. However a gene encoding CpSUB1, a subtilisin-like serine protease is present. In this study, we cloned the CpSUB1 genomic sequence and expressed and purified the recombinant prodomain. Reverse transcriptase-PCR analysis of RNA from *C. parvum*-infected HCT-8 cells revealed that CpSUB1 is expressed throughout infection *in vitro*. In immunoblots, antiserum to the recombinant CpSUB1 prodomain reacted with 2 major bands of ~64 kDa and ~48 kDa in *C. parvum* lysates and proteins “shed” during excystation. In immunofluorescence assays, the antiserum reacted with the apical region of sporozoites and merozoites. The recombinant prodomain inhibited protease activity and processing of recombinant gp40/15 by *C. parvum* lysates but not by furin. Since prodomains are often selective inhibitors of their cognate enzymes, these results suggest that CpSUB1 may be a likely candidate for the protease activity in *C. parvum* and for processing gp40/15. Importantly, the recombinant prodomain inhibited *C. parvum* infection of HCT-8 cells. These studies indicate that CpSUB1 plays a significant role in infection of host cells by the parasite and suggest that this enzyme may serve as a target for intervention.
Title:
Microarray Analysis of Cryptosporidium parvum-Infected Epithelial Cells Uncovers Modifications of the Host Cell Cytoplasmic Membrane

Authors:
Yi-Lin Yang, Myrna Serrano, Gregory Buck, Giovanni Widmer

Presented by:
Yi-Lin Yang

Departments:
Department of Biomedical Sciences, Division of Infectious Diseases, Cummings School of Veterinary Medicine; Center for the Study of Biological Complexity, Virginia Commonwealth University

Abstract:
Understanding the complexity of host-pathogen interaction in cryptosporidiosis is central to developing new therapeutic strategies. Cryptosporidiosis can be a severe infection in children and immuno-compromised individuals. We used microarray technology to analyze the change in gene expression in a human intestinal epithelial cell line (HCT-8) in response to C. parvum infection. Due to the heterogeneous nature of C. parvum infected monolayer, it is difficult to differentiate transcriptional changes occurring directly in response to the infection and those resulting from the perturbation of the monolayer induced by the infection. Facilitated by flow cytometry, infected and uninfected cells from the same monolayer were analyzed to identify genes which are differentially transcribed as a direct consequence of the presence of intracellular C. parvum. Over-representation of Protease Activated Receptor-2 (PAR2), a transmembrane protein, was first identified by comparing the transcriptome of infected monolayer. Transcriptional regulation of this gene was further validated in infected cells using reverse transcriptase PCR. Immunofluorescence analysis revel that over-expressed PAR2 is located with a well-defined region of host cell membrane which surrounds the intracellular stages of the parasite. By directly comparing transcriptional changes in infected and uninfected cells originating from a same cell monolayer, genes encoding glycoprotein were found to be significantly over-represented among up-regulated genes. Glycoproteins expressed on C. parvum infected cells were further characterized using fluorescently labeled lectins. With this approach we determined N-acetyl-D-galactosamine to be a common carbohydrate residue on infected cells. Glycoproteins decorated with this carbohydrate were present on the surface of the host cell membrane as well as inside the cells in proximity of the intracellular parasites. These observations illustrate the impact of the parasite on the composition of the cytoplasmic membrane of infected epithelial cells.
Title:
Utility of Pyrosequencing of the Bacterial 16S rRNA Genes and Computational Analysis in Studying Alterations in the Gut Microbiota During Cryptosporidiosis

Authors:
Sean Nealy, Veeraraghavan Balaji, Sitara Rao Ajjampur, Rajiv Sarkar, Gagandeep Kang, Roberta O'Connor, Honorine Ward, Anne Kane, Debra Poutsiaka

Presented by:
Sean Nealy

Departments:
Department of Medicine, Tufts University School of Medicine; Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India; Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
The microbiota of the gut play a role in many of the body’s functions and in numerous diseases. Fecal samples from three pediatric patients enrolled a prospective cohort study of cryptosporidiosis in Vellore, South India were used to show changes in the gastrointestinal (GI) microbiota before, during, and after infection. DNA was isolated from stool samples, and the genes encoding bacterial 16S rRNA (16S rDNA) were amplified and pyrosequenced. The resulting sequences were trimmed of poor quality sequences, aligned according to the RDP Classifier and then clustered according to genetic relatedness. Subsequently indices describing the ecology of the gut microbiota were analyzed. We found differences in richness over the course of cryptosporidiosis in two of the three patients. In these patients, richness increased during cryptosporidiosis and fell after recovery. Changes in diversity paralleled these results, with an increase during illness and a subsequent decrease afterwards. There were also changes in the relative proportions of the major phyla represented in the gut microbiota but these changes followed no pattern. A larger study will be necessary to provide conclusions about gut microbiota alterations during cryptosporidiosis.

We conclude that pyrosequencing fecal bacterial 16S rDNA and computational analysis employing the RDP Pipeline are useful tools in the study of the disruptions in the GI microbiota over the course of an illness.
Title:
Respiratory Cryptosporidiosis in Ugandan Children

Authors:
Siobhan Mor, James Tumwine, Grace Ndeezi, Maheswari Srinivasan, Deogratias Kaddu-Mulindwa, Saul Tzipori, Jeffrey Griffiths

Presented by:
Siobhan Mor

Departments:
Department of Biomedical Sciences, Cummings School of Veterinary Medicine; Department of Public Health and Community Medicine, Tufts University School of Medicine; Departments of Pediatrics and Child Health and of Medical Microbiology, Makerere University, Kampala, Uganda; Gerald J. and Dorthy R. Friedman School of Nutrition Science and Policy

Abstract:
Respiratory infection with Cryptosporidium is recognized as a late-stage complication of intestinal cryptosporidiosis in HIV/AIDS patients. Respiratory signs and symptoms are also common in otherwise healthy children with intestinal cryptosporidiosis, suggesting that respiratory infection may be more universal than currently accepted. In this cross-sectional study, we recruited children aged 9–36 months presenting with diarrhea to Mulago Hospital in Kampala, Uganda. Children with Cryptosporidium-positive and -negative stools (ratio 4:1) were selected for further evaluation, including sputum induction (in those with cough or unexplained respiratory signs) and collection of saliva and blood. Sputum samples were subjected to comprehensive bacteriological testing, and both sputum and saliva were tested for Cryptosporidium by nested-PCR. Of 926 fecal samples screened, 116 (12.5%) were Cryptosporidium positive. Cough or other respiratory signs were present in 73.3% of stool-positive children, compared to 66.5% of stool-negative children (p=0.148). Seventeen of 48 (35.4%) sputum samples from stool-positive children were positive for Cryptosporidium. Sixteen of the 17 children with confirmed respiratory cryptosporidiosis were HIV-seronegative and 10/17 (58.8%) children were normally nourished. None of the 12 sputum specimens tested from stool-negative children were Cryptosporidium positive (p=0.013 compared to stool-positive children). Parasite DNA was only detected in 2/103 (1.9%) saliva samples (p<0.0001 compared to sputum). These findings are novel and suggestive of an under-appreciated site of infection that may have important implications given the potential for respiratory transmission.
Title:
Microsporidiosis and Malnutrition in Ugandan Children with Persistent Diarrhea

Authors:
Siobhan Mor, James Tumwine, Grace Ndeezi, Saul Tzipori

Presented by:
Siobhan Mor

Departments:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine; Department of Pediatrics and Child Health, Mulago Hospital, Makerere University Medical School, Kampala, Uganda

Abstract:
The microsporidian fungus, Enterocytozoon bieneusi, causes significant morbidity in HIV-positive adults due to persistent diarrhea, intestinal malabsorption and wasting. Although infection is common in children, the impact of microsporidiosis on the nutritional health of this vulnerable population has not been thoroughly examined. In this cross-sectional study, we investigated the effect of microsporidiosis on growth rates of Ugandan children aged <60 months with persistent diarrhea. Simple and multiple linear regression was employed to test whether the rate of change in weight or height differed in children with and without microsporidiosis. After simultaneously adjusting for the impact of sex, HIV and concurrent Cryptosporidium infection, microsporidiosis was independently associated with reduced weight gain but not linear growth (p=0.014 and p=0.151, respectively). The predicted growth trajectory of children with microsporidiosis was such that, by age 5, these children were approximately 1.3kg lighter than children without microsporidiosis. While a causal role of E. bieneusi cannot be implied from this cross-sectional data, we present evidence that microsporidiosis is associated with reduced weight gain in children with persistent diarrhea. Further longitudinal studies are required to establish the direction of this association and to determine whether these sub-normal growth rates are followed by catch-up growth.
Title:
Cryptosporidiosis-Related Hospitalization in the US Elderly Population

Authors:
Siobhan Mor and Elena Naumova

Presented by:
Siobhan Mor

Departments:
Department of Biomedical Sciences, Cummings School of Veterinary Medicine; Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
The protozoan, Cryptosporidium spp., is a major cause of gastrointestinal illness worldwide. Though cryptosporidiosis has been a nationally notifiable disease in the USA since 1995, surveillance estimates are greatly undermined by failures to diagnose infection in the healthcare setting as well as report identified cases to health authorities. It is widely believed that immune senescence leads to enhanced vulnerability to cryptosporidiosis in the elderly; however this is not generally supported by surveillance data. The Centers for Medicare and Medicaid Services (CMS) databases present a novel means to investigate the burden of cryptosporidiosis in the elderly US population without the bias of underreporting. We abstracted all records (n=1304) containing a diagnosis of cryptosporidiosis-related (CR) illness in persons aged ≥65 years for the years 1991–2004. Annual rates of CR hospitalization were calculated and compared to surveillance data published by the CDC. Comorbidity and outcome of hospitalization was also assessed. CR hospitalizations increased during the study period, from 0.15 to 0.39 cases per 100,000 elderly per year, most likely due to increased awareness and testing. Comparison between the annual rates of CR hospitalization and CDC surveillance data revealed considerable state-to-state variation. Annual rates of CR hospitalization increased with age, such that the rate in persons aged >85 years more than doubled that in persons aged 65–74. Fluid and electrolyte imbalance was a common comorbidity while cancer was surprisingly infrequent. In-hospital death occurred in 5.4% of cases; the highest case fatality was in persons aged >85 (7.1%) and those with HIV (12.8%).
Title:
From Broad Host Range to Isogenic Prison: Genotypic Plasticity in Experimentally Evolved Strains of Legionella

Authors:
Alexander Ensminger and Ralph R. Isberg

Presented by:
Alexander Ensminger

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
Legionella pneumophila is a Gram-negative facultative intravacuolar bacterial pathogen. The primary natural reservoir of L. pneumophila is likely freshwater amoebae, where the bacteria replicate intracellularly in a specialized vacuole that avoids interaction with the lysosomal network, at least during the early stages of replication. L. pneumophila exhibits a strikingly broad host range, and the replication of the bacteria in mammalian macrophages is remarkably similar to its replication in its natural amoebal hosts. We have developed an experimental evolution experiment designed to measure the genomic and phenotypic plasticity of bacteria restricted to a single host background for several hundred generations. Multiple lines of Legionella were serial batch cultured through isogenic populations of cultured host cells. After this prolonged passage, we observe increased fitness to the adapted host environment in multiple lines as well as other surprising secondary phenotypes. The axenic growth rate and host range of each adapted strain has also been examined. Through Illumina-based whole genome resequencing of these lines, we have identified the mutations acquired by each adapted strain. Intriguingly, many of these mutations are in bacterial pathways that may impinge on recognition by the host innate immune system. Using multiple genotyping platforms, the allelic frequency of each mutation has also been determined for intermediate time points throughout the experiment. This approach allows for each phenotypic acquisition to be retrospectively correlated to the dynamics of mutation emergence and subsequent fixation or loss. These results provide unique insight into the intracellular lifestyle of this important pathogen as well as begin to address whether mammalian pathogens are optimally adapted to the mammalian host.
Title: Activation of NF-κB During *Legionella pneumophila* Infection

Authors: Eva Haenssler, Vicki Losick, Man-Yu Moy, Ralph R. Isberg

Presented by: Eva Haenssler

Department: Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract: *Legionella pneumophila* is a facultative intracellular bacterium that after inhalation of contaminated water sources replicates in human alveolar macrophages thereby causing severe pneumonia known as Legionnaire’s disease. Intracellular replication of the pathogen requires the formation of a membrane bound vacuole by recruitment of ER-derived vesicles. This process is dependent on the presence of the Dot/Icm type IV secretion system that allows translocation of bacterial effector proteins into the host cytoplasm. *L. pneumophila* effector proteins have been shown to manipulate a variety of host cell processes in order to generate a beneficial environment for growth.

Since for an intracellular pathogen host cell survival is of crucial importance for replication, host cell death pathways are one major target of translocated substrates. Previous research has shown that one strategy pursued by the bacterium is the induction of anti-apoptotic genes regulated by the nuclear factor κB (NF-κB) transcription factor. At low bacterial loads, this induction depends on the Dot/Icm system, so that consequently a screen with a library of translocated substrates was performed to identify proteins that serve as inducers of NF-κB. In addition to already characterized proteins that were shown to act as mild inducers, two so far not described strong inducers of NF-κB were found. Subsequent work on one of the proteins named LnaB led to the identification of respective functional domains. Furthermore, the influence of this protein on bacterial growth and intracellular replication as well host cell vesicle trafficking and death was examined. As a future goal the mechanism on how LnaB contributes to the activation of NF-κB will be uncovered by searching for host cell interaction partners. Activation of NF-κB does not necessarily involve interference with pathways downstream of pattern recognition receptors, like Nod proteins or Toll-like receptors, it might also be the result of indirect mechanisms like perturbation of ER homeostasis or alteration of the actin cytoskeleton.
Title:
The Role the Human DUSPs in *Legionella pneumophilia* Infection

Authors:
Aisling Dugan and Ralph R. Isberg

Presented by:
Aisling Dugan

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
*Legionella pneumophilia* is a gram-negative bacterium that naturally infects the fresh water amoeba. *L. pneumophila* is also the etiological agent of Legionnaire’s Disease, a severe atypical pneumonia most often found in elderly or immunocompromised patients, which is caused by inhaling bacteria containing water droplets. The infectious process begins when a bacterium is engulfed by a human immune cell in the lung called an alveolar macrophage. Inside this host cell, the bacterium replicates and avoids degradation by the immune system. Our research is focused on identifying the factors, both host cell and bacterial, that are critical for *Legionella* to replicate and disseminate. Our data revealed that the members of the host cell dual specificity phosphatase (DUSP) family are highly upregulated after infection. In the cell, DUSPs function by silencing the mitogen activated protein kinases (MAPKs). MAPKs are intracellular enzymes that help to link extracellular stimuli into a biological response. We hypothesize that turning MAPKs off may be critical to the survival of intracellular pathogen by inactivating the host cell anti-microbial response. The experiments described herein are aimed to (1) determine if DUSP genes are necessary for infection, (2) elucidate the effect DUSP expression and activity has on the cell, and (3) identify which bacteria protein(s) activate the DUSP response.
Title:
Mechanisms of Translocation of *Legionella pneumophila* Effectors via the Dot/Icm Type IVB Secretion System

Author:
Whitney Amyot

Presented by:
Whitney Amyot

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:

*Legionella pneumophila* is an intracellular pathogen that avoids fusion with lysosomal compartments and forms a replication vacuole derived from rough endoplasmic reticulum. Biogenesis and growth within the *Legionella*-containing vacuole requires a functional type IVB secretion system (T4SS) known as the Dot/Icm complex. Considerable research is underway to identify and characterize upwards of 250 bacterial effector proteins that are translocated through the Dot/Icm complex. However, the mechanism by which effector proteins are transported through the Dot/Icm system to the phagosomal surface or the host cytoplasm has yet to be elucidated.

A primary question of the mechanisms of translocation is whether this process requires unfolding of the translocated protein. To monitor the folding state of proteins during translocation, we constructed protein fusions of various *L. pneumophila* effectors to the rapidly and tightly folding dihydrofolate reductase (DHFR) protein. Fusions to DHFR prevented the translocation of nearly all the effectors studied, including a 50 amino acid carboxy-terminal tag of an effector protein, suggesting that tightly folded proteins may not be able to pass through the Dot/Icm complex. Surprisingly, a DHFR fusion to the effector protein Lpg1798 is still translocated at significant levels. Current studies are underway to characterize the folding or other inherent properties of the protein that either allow or inhibit translocation of a DHFR fusion.

Interactions of *L. pneumophila* proteins IcmS and IcmW are believed to serve as adaptor proteins for the translocation of a large subset of Dot/Icm effector proteins. We have identified varying levels of dependence on IcmS for translocation within the effectors studied and have some evidence that this phenotype is unrelated to direct binding of IcmS to the effector protein. Of particular interest, it was found that the translocation defect in a DHFR-effector fusion is exacerbated in an icmS deletion mutant. These data taken together suggest a role for IcmS in facilitating a translocation competent Dot/Icm complex.

Since the LCV is surrounded by rough ER vesicles within 5 minutes after uptake, a process that requires Dot/Icm effectors, it is likely that translocation in *Legionella* occurs at a high rate. In an infection time course, a kinetic curve demonstrated a steady increase in translocation until it reached steady state levels approximately 1 hour after infection. Additional studies using FRET to visually monitor translocation in real time are underway to further characterize the kinetics of translocation immediately upon contact between *L. pneumophila* and the host cell, as well as throughout the infection process.
Title:
Identification and Characterization of Effectors of the *Legionella Pneumophila* Type IVB Secretion System

Author:
Andrew Hempstead

Presented by:
Andrew Hempstead

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
*Legionella pneumophila*, the causative agent of Legionnaires’ disease, is a Gram-negative bacterium which is able to survive and replicate within both protozoan and mammalian hosts. Intracellular survival is accomplished by the translocation of effector proteins through the Dot/Icm type IVB secretion system. These effectors promote the biogenesis of a replication vacuole that bypasses phagosome-lysosome fusion, and alters the host innate immune response. Only one of the 190 known effector proteins has been shown to be necessary for replication within macrophages. A recent transposon mutagenesis screen has identified multiple genes likely important for intracellular replication, including several known and putative effectors. Four putative effectors, identified by this screen were chosen for further study and assayed for translocation as well as localization within host cells. Future studies, using in frame deletions, will determine the importance of each of these effectors to the intracellular survival of this pathogen.
Title:
Purinergic Signaling and Immune Modulation at the Schistosome Surface

Authors:
Rita Bhardwaj and Patrick Skelly

Presented by:
Rita Bhardwaj

Department:
Department of Biomedical Sciences, Cummings School of Veterinary Medicine

Abstract:
Schistosomes are human parasitic flatworms that constitute an important public health problem globally. The parasites live for years, sometimes decades, in what is putatively a very hostile environment – the blood of vertebrates – yet they seem to elicit little if any protective reaction from two of the host’s major defensive systems: the hemostatic system and the immune system. We hypothesize that this is because schistosome nucleotide metabolizing ecto-enzymes (NMEEs, alkaline phosphatase (SmAP), ecto-phosphodiesterase (SmPDE) and ecto-ATP-diphosphohydrolase (SmATPDase)), among a small subset of proteins expressed on the parasite surface membranes, dampen host pro-inflammatory and pro-thrombotic purinergic signaling mechanisms. In this way, these surface enzymes attenuate the host’s ability to focus damaging thrombotic and immunological mediators in the parasite’s vicinity (Bhardwaj and Skelly, 2009). In this work, we show that the expression of all 3 NMEE genes is upregulated following vertebrate host invasion and that all are located in the tegument, by immunofluorescence and immuneEM. RNAi treatment targeting each NMEE gene results in potent suppression of gene expression, as determined by quantitative real-time PCR and by western analysis. The viability of suppressed versus control parasites is similar in culture but is significantly diminished in vivo. Finally, we show that, unlike parasites whose SmAP and SmPDE genes are suppressed, parasites whose SmATPDase gene is suppressed are significantly impaired in their ability to catabolize the potent pro-inflammatory molecule, ATP. These data are consistent with the idea that some NMEEs provide an important immunomodulatory role for schistosomes within their hosts.
Title:
Dendritic Cell IL-23 and IL-1 Production in Response to Schistosome Eggs Induced Th17 Cells in a Mouse Strain Prone to Severe Immunopathology

Authors:
Mara Shainheit, Patrick Smith, Lindsey Bazzone, Andrew Wang, Laura Rutitzky, Miguel Stadecker

Presented by:
Mara Shainheit

Departments:
Departments of Pathology and of Immunology, Tufts University School of Medicine

Abstract:
Infection with schistosomes results in a CD4 T cell-mediated inflammatory reaction against parasite eggs that varies greatly in magnitude both in humans as well as in mice. In the murine disease, the severe form of immunopathology correlates with high levels of interleukin 17 (IL-17). We now report that live schistosome eggs stimulate dendritic cells from high pathology-prone CBA mice to produce IL-12p40, IL-6 and TGF-β, whereas those from low pathology-prone BL/6 mice only make TGF-β. Moreover, egg stimulated dendritic cells plus naïve CD4 T cells from CBA mice resulted in increased levels of IL-6, IL-23, IL-1β, as well as IL-17 and the chemokines CXCL1, CXCL2 and CCL2, whereas similarly treated BL/6 cell co-cultures instead expressed higher IL-4, IL-5, IL-10 and the transcription factor Foxp3. Neutralization of IL-23 and IL-1, but not of IL-6 or IL-21, profoundly inhibited egg-induced IL-17 production in the CBA co-cultures. Conversely, stimulation with schistosome eggs in the presence of exogenous IL-23 and IL-1β induced BL/6 cells to make IL-17. These findings identify IL-23 and IL-1 as critical host factors that drive IL-17 production, and suggest that parasite recognition followed by a genetically determined innate pro-inflammatory response induces the development of Th17 cells and the outcome of immunopathology in schistosomiasis.
Title:
Genetic Control of Severe Egg-Induced Immunopathology and IL-17 Production in Murine Schistosomiasis

Author:
Patrick Smith

Presented by:
Patrick Smith

Department:
Department of Immunology, Tufts University School of Medicine

Abstract:
Infection with the trematode parasite *Schistosoma mansoni* results in a distinct heterogeneity of disease severity, both in humans and in an experimental mouse model. Severe disease is characterized by pronounced hepatic egg-induced granulomatous inflammation in a proinflammatory cytokine environment, while mild disease corresponds with reduced hepatic inflammation in a Th2 skewed cytokine environment. This marked heterogeneity indicates that genetic differences play a significant role in disease development, yet little is known about the genetic basis of dissimilar immunopathology. To investigate the role of genetic susceptibility in murine schistosomiasis, quantitative trait loci (QTL) analysis was performed on an F2 progeny derived from SJL/J and C57BL/6 mice, which develop severe and mild pathology, respectively. We now show that severe liver pathology in 7 week-infected F2 mice significantly correlated with an increase in the production of the proinflammatory cytokines IL-17, IFN-γ and TNF-α by schistosome egg antigen-stimulated mesenteric lymph node cells. QTL analysis identified several genetic intervals controlling immunopathology as well as IL-17 and IFN-γ production. Egg granuloma size exhibited significant linkage to two loci, D4Mit203 and D17Mit82, both of which were inherited in a BL/6 dominant manner. Furthermore, a significant reduction of hepatic granulomatous inflammation and IL-17 production in interval specific congeneric mice demonstrated that the two identified genetic loci have a decisive effect on the development of immunopathology in murine schistosomiasis.
Title:
Improved Diagnostic Accuracy of Urine Filtration and Dipstick Tests for Urinary Schistosomiasis via Quadruplicate Screening of a Lightly-Infected Population of Ghanaian School Children

Author:
Karen Kosinski

Presented by:
Karen Kosinski

Department:
Department of Civil and Environmental Engineering, Tufts University School of Engineering

Abstract:
Two testing methods, the use of reagent dipsticks for hematuria (blood in urine) and urine filtration for Schistosoma haematobium eggs, were evaluated for their sensitivity and specificity in diagnosing S. haematobium, or urinary schistosomiasis, infections. In Adasawase, Ghana, a rural community in the Eastern Region, school children aged 8 to 18 years (n=255) provided urine samples on four separate screening occasions and the cumulative prevalence of schistosomiasis in the population was then estimated. Overall, 39.7% of girls and 53.3% of boys presented with eggs at least once, but none of the girls and only five boys presented with both eggs and hematuria during all four screenings. The data suggest that true infection status can be accurately assessed only if multiple urine samples per person are tested. With respect to testing method, many children presented with eggs but without hematuria, or with hematuria but without eggs, on a given screening day. Thus, testing via only one method may cause underestimation of schistosomiasis prevalence. Here, when each child was screened four times, the mean sensitivity of both types of urine test improved by at least 33.6%, but new schistosomiasis cases were still detected even at the fourth screening, albeit with a diminishing rate of return. Finally, the majority of lightly-infected children (< 50 eggs/10ml urine, by maximum egg count) were egg-negative on at least half of the screening days demonstrating, particularly in lightly-infected populations, the benefit of multiple days of testing. This study is part of a larger research project which emphasizes primary prevention techniques with an alternative water play area currently being assessed for efficacy of decreasing disease transmission.
Title:
Correlates of HIV-1 Viral Suppression in a Twelve Month Follow-up Study of HIV-Positive Drug Users Receiving Antiretroviral Therapy in Hanoi, Vietnam

Authors:
Michael Jordan, Christine Wanke, Hanh La, Heidi Sheehan, Hien Duc Nguyen, Lien Trinh, Dang Dong, Rony Barbara, Alice Tang

Presented by:
Michael Jordan

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Department of Public Health and Community Medicine, Tufts University School of Medicine; National Institute of Infectious and Tropical Diseases, Hanoi, Vietnam

Abstract:

Background: Fifty-six percent of all reported HIV infections in Vietnam are in drug users (DUs) and treatment of DUs is a focus of national antiretroviral therapy (ART) treatment programs. This analysis assesses substance use and clinical determinants of HIV suppression in a cohort of male DUs receiving ART at a large urban clinic in Hanoi, Vietnam.

Methods: Correlates of viral suppression were studied in 100 DUs receiving ART for at least 6 months. Clinical, sociodemographic, and laboratory analyses were assessed at study entry and at 12 months. Variables included sociodemographics, substance use history, and ART adherence: 30 day visual analogue scale (VAS) and Likert scale. CD4+ cell count and HIV RNA were obtained at entry and at 12 months. Viral load suppression defined as RNA<1000 copies/ml. T-tests for continuous variables and chi-square tests for categorical variables were performed.

Results: Mean age 29.9 ± 4.9 years. Twenty-three percent reported previous incarcerated and 48% reported active drug use. ART duration at entry (6.2 – 85.7; median 13.6 months) 73% achieved viral suppression at entry. Correlates of suppression at entry included self-reported >95% adherence (90% vs. 70%, p<0.01); concurrent use of trimethoprim/sulfamethoxazole (85% vs. 56%, p<0.01). Current or previous tuberculosis associated with viral non-suppression (41% vs. 15%, p=0.006). At 12 months, 90 remained in the study; Dead (2); Prison (2); lost-to-follow-up (3); transferred care (3). Correlates of suppression included: Likert scale (p=0.025) and switch to boosted protease inhibitor in patients without suppression at entry. Current substance use was not associated with viral non-suppression.

Conclusions: Successful ART treatment outcomes in a population of Vietnamese DUs are documented. Rates of suppression are comparable to other international populations. The 30% of patients without viral suppression highlights the need for population based surveillance strategies to assess emergence of drug resistance and associated factors in settings where access to viral load and drug resistance testing is limited.
Title:
Variability in the Pharmacokinetics of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) in TB/HIV Co-Infected Ghanaian Patients

Authors:
Awewura Kwara, Margaret Larney, Isaac Boamah, Naser Rezk, Joseph Oliver-Commey, Ernest Kenu, Angela Kashuba, Michael Court

Presented by:
Michael Court

Departments:
Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine; Warren Alpert Medical School of Brown University; University of North Carolina at Chapel Hill; University of Ghana Medical School and Korle-Bu Teaching Hospital, Accra, Ghana

Abstract:

Background: There are limited data on the pharmacokinetics (PK) of generic nucleoside reverse transcriptase inhibitors (NRTIs) in populations in Africa, where they are widely used. We evaluated the PK profiles of lamivudine (3TC), zidovudine (ZDV) and stavudine (d4T) as well as the determinants of interindividual variability.

Methods: Thirty Ghanaian HIV/TB co-infected patients on rifampin-containing TB therapy and trimethoprim-sulfamethoxazole were enrolled and treated with efavirenz plus 3TC and ZDV (Duovir®) or 3TC and d4T. Steady-state samples were obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours post-dosing. Drug levels were determined by a validated HPLC method. Direct sequencing of the UDP-glucuronosyltransferase 2B7 gene exon 2 was performed. The relationship between patient covariates, UGT2B7 genotype, and PK data were assessed by t-test, ANOVA and linear regression. PK data were log-transformed or rank-transformed as appropriate to achieve data normality and equal variance. Results are expressed as mean values (SD).

Results: Twenty-seven patients (74% males) with complete data were included in this analysis. The AUC [coefficient of variation] of 3TC (n=27) was 5843 (2581) h*ng/mL [44%], ZDV (n=16) was 3368 (2747) h*ng/mL [82%] and d4T (n=11) was 865 (223) h*ng/mL [26%]. 3TC AUC was significantly lower in patients who received 3TC with ZDV compared to those who received 3TC and d4T (5474 vs. 6911 h*ng/mL, P=.031) but weight-normalized apparent oral clearance (CL/F) were similar (459.2 vs. 406.0, P=.824). Compared with non-carriers, carriers of the UGT2B7*1c (c.735A>G) had lower mean ZDV AUC (2160 vs. 4997 h*ng/mL, P=.029), shorter plasma half-life (4.0 vs. 12.2 hours, P=.030), and higher CL/F (47.6 vs. 16.2 mL/min/kg, P=.004). We did not find sex differences in PK for ZDV and 3TC in this small population. However, d4T CL/F was higher in females than males (8.8 vs. 6.0 mL/min/kg, P=.014). Age and BMI were not associated with the PK of any NRTIs. All evaluated patients had suppressed plasma HIV-1 levels within 24 weeks of therapy.
**Conclusions:** There is significant variability in pharmacokinetic profile of commonly used NRTIs in Ghanaian HIV/TB co-infected patients on TB therapy but no difference in short-term virologic suppression. Also, we found a novel association between UGT2B7 genetic variation and ZDV PK. The relationships between variable PK profiles, intracellular concentrations, clinical effect and long-term toxicity need to be evaluated.
Title:
Urogenital Tuberculosis

Author:
Sreevani Gudiseva

Presented by:
Sreevani Gudiseva

Department:
North Shore Medical Center

Abstract:

Learning Objectives: This case is used to raise awareness of and formulate a diagnostic approach to this important entity.

Case Presentation: Sixty five year old male came in with the complaint of weakness for 6 months. He complained of back pain for 3 weeks, 8/10 in severity, no specific aggravating and relieving factors. Complained of fever, vomiting for the past week and intermittent dry cough. Denied chest pain, palpitations, SOB, hemoptysis, night sweats or weight loss.

Past medical history includes diabetes, hypertension, peripheral neuropathy, BPH, hydronephrosis, and renal insufficiency. No history of tuberculosis exposure. No allergies. Quit smoking 10 years ago, nonalcoholic.

Physical exam revealed a lethargic male with a temperature of 102.4, BP of 140/60, pulse rate of 80 and saturation was 96%. Auscultation revealed occasional crackles on the right side, regular heart sounds. Genital exam revealed non tender cystic mass in the right testis.

Labs revealed hemoglobin 9.2, hematocrit 27, white count 12, platelets 634, differential count was neutrophils 76.5%, lymphocytes 15.5%, monocytes 9.5%, basophils 0.1% and absolute neutrophil count was 9.2, sodium 118, potassium 7.1, chloride 89, bicarb 99, BUN 214 and creatinine was 15.4, glucose 393. Chest X-Ray normal.

He was admitted to ICU and dialyzed. He continued to have fevers with negative cultures. TEE was normal. CT scan of the abdomen and pelvis revealed bilateral hydroureteronephrosis and tiny bilateral renal lesions. CT scan of the chest revealed evidence of prior granulomatous disease, calcified mediastinal nodes, calcified right middle lobe granuloma and a non calcified left upper lobe nodule. PPD came back positive.

Testicular ultrasound revealed irregularly marginated, loculated fluid collection in the right scrotum. Bladder biopsies revealed acute and chronic cystitis. Biopsy of the testicular fluid collection revealed granulomatous
process with necrosis. Both fluid and urine were sent for AFB cultures which were positive. Patient was started on the four drug regimen with rifampicin, isoniazid, pyrazinamide, and ethambutol.

**Discussion:** Genitourinary tuberculosis is the most common extrapulmonary manifestation and represents 2-4% of the tuberculosis cases. The spread of tuberculosis to epididymis occurs hematogenously or by retrocanalicular descent of organisms from the infected prostate. Distal spread through the genitourinary tract from a renal source is possible. By the time of diagnosis, the primary source of pulmonary infection may be inactive or calcified. Tuberculosis may cause chronic epididymitis and epididymo-orchitis. Tuberculous granulomas may develop within the testes and epididymis, rarely complicated by abscesses and discharging sinuses. Prostatitis, thickening of the scrotal wall and tunica albuginea, as well as moderate hydrocele may be observed. FNAC is the first choice investigation which provides a diagnosis, preventing unnecessary orchidectomy. Treatment of genital tuberculosis is the four drug regimen consisting of rifampicin, ethambutol, isoniazid, and pyrazinamide.
Title: Sputum Vital Stain Microscopy to Predict Sputum Culture Results and Infectiousness

Authors: Marjory Bravard, Jonathan Sherman, Laura Martin, Louis Grandjean, Teresa Valencia, Rosario Montoya, Robert Gilman, Carlton Evans

Presented by: Marjory Bravard

Departments: Tufts University School of Medicine; Laboratorio de Investigación y Desarrollo (LID), Departamento de Microbiología, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru; Asociación Benéfica Prisma, Lima, Peru; Johns Hopkins Bloomberg School of Public Health; Wellcome Centre for Clinical Tropical Medicine; Department of Infection and Immunity, Imperial College, London, United Kingdom

Abstract:

Background: Assessing whether patients remain an infection risk requiring isolation after commencing tuberculosis therapy is difficult because decisions are based on culture results from sputum samples collected many weeks previously. We therefore optimized and validated fluorescein diacetate (FDA) vital staining for predicting tuberculosis culture results.

Methods: A protocol was optimized to stain live but not dead bacilli in decontaminated sputum samples dried onto microscopy slides using fluorescein diacetate staining and standard fluorescence microscopy. The reliability of fluorescein diacetate slide microscopy in predicting quantitative culture results was compared to that of auramine microscopy. Fluorescein diacetate was assessed in 2 blinded experiments:

1) VALIDATION: Combination dilutions of live and sterilized, boiled sputum from untreated patients were mixed in different proportions
2) EVALUATION: Sequential sputum samples from treated patients were collected before and after 3, 6, and 9 days of first-line tuberculosis treatment.

Results: Fluorescein diacetate slide microscopy took 40-60 minutes and required basic skills and only a microscope. Quantitative culture took 3-6 weeks and required a biosafety cabinet, centrifuge, vortex, incubator and moderate laboratory expertise. In VALIDATION experiments, FDA-microscopy accurately reflected the proportion of live tuberculosis in each sample. In EVALUATION experiments, FDA-microscopy reliably predicted quantitative culture results (r²=0.77). In contrast, auramine microscopy did not reliably predict quantitative cultures (r²=0.33 before treatment and r²=0.26 during treatment).

Conclusions: As compared to weeks of information lag when using quantitative cultures, fluorescein diacetate microscopy reliably predicts future cultures results in minutes, allowing for a dynamic, real-time approach to infection control.
Title:
Working Elephants as Possible Intermediaries for Disease Transmission Between Domestic Livestock and Wild Ungulates in Chitwan, Nepal

Authors:
Gretchen Kaufman, Sarad Paudel, Kamal Gairhe, Kristi Delaski, Karin Hamilton, Louise Maranda, Christine Jost, Sean Griffin

Presented by:
Gretchen Kaufman

Departments:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine; Institute of Agriculture and Animal Science, Rampur, Nepal; HMG Department of National Parks and Wildlife Conservation, Sauraha, Nepal

Abstract:
There are approximately 150 to 200 captive Asian elephants (Elephas maximus) in the buffer zone area bordering Chitwan National Park, in the Terai Arc of Nepal. Elephants are used by the tourism industry for jungle rides and by the government for forest management and tourism. A captive elephant breeding center is also operated by the government in the same buffer zone area. These elephants regularly come in contact with domestic livestock in the buffer zone and with wild ungulates in the National Park including gaur, hog deer, barking deer, chital deer, sambar deer, one-horned rhinoceros and wild Asian elephants. A series of animal health and demographic studies were conducted in the Chitwan buffer zone by Tufts University and the Institute of Agriculture and Animal Science, in collaboration with the government of Nepal, Department of National Parks and Wildlife Conservation. Health data collection for these studies included location determination using a Garmin global position system (GPS). GPS was also used to establish the captive elephant’s ranges by accompanying representative elephants on their grazing and working routes in two seasons, and recording location every 200 meters. A database was constructed with ArcGIS, combining the elephant range data and the health surveillance location data. This database illustrates the potential role that the working elephants could play in transmission of disease between domestic livestock and the wild ungulates protected within the park and establishes a basis for future modeling of disease transmission in the Chitwan region.

Poster originally presented at the Annual Conference of the Wildlife Disease Association, Storrs, CT, August 6-10, 2006.
Title:
Controlling Rabies in Nepal – An International Collaborative Research and Service Program at Tufts Cummings School of Veterinary Medicine

Author:
Gretchen Kaufman

Presented by:
Gretchen Kaufman

Departments:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine

Abstract:
Nepal is a small, landlocked country situated between India and Tibet, with a human population of approximately 27 million. Nepal has one of the highest reported per capita rates of human rabies deaths in the world. As is the case with many other developing countries, the persistence of this disease is centered on domestic dogs as the primary disease reservoir. This disease is perpetuated by several factors, including the lack of routine vaccination among pet dogs, the existence of a large uncontrolled and unvaccinated stray dog population, lack of rabies prevention education and other socio-economic and political challenges. Tufts international veterinary faculty and students have been working in Nepal since 2001 to help develop the capacity and to support the political means to combat rabies on a national basis. We have partnered with key veterinary educational institutions (Institute of Agriculture and Animal Science (IAAS), Himalayan College of Agricultural Sciences and Technology), as well as with key non-governmental organizations (Kathmandu Animal Treatment Centre (KAT), National Zoonosis and Food Hygiene Research Centre) to address fundamental issues that impede controlling rabies in Nepal. Our efforts have been focused on developing the capacity for rabies public education, dog rabies vaccination and surgical dog sterilization among Nepal’s professional veterinary community and on developing sustainable options for implementing community-based rabies control programs. Faculty have contributed to training initiatives, curriculum development, program development and support, and policy negotiations with key stakeholders. Tufts’ students participate through mentored summer research projects conducted either in association with the IAAS Rabies prevention program in Chitwan or in association with the KAT Centre in Kathmandu.
Title:
Tuberculosis in Wildlife, Livestock and People in Nepal – An International Research and Service Program at Tufts Cummings School of Veterinary Medicine (Umbrella Program Poster)

Authors:
Gretchen Kaufman, Jean Mukherjee, Donna Akiyoshi

Presented by:
Gretchen Kaufman

Departments:
Department of Environmental and Population Health and of Biomedical Sciences, Cummings School of Veterinary Medicine

Abstract:
Nepal is a small, landlocked country situated between India and Tibet, with a human population of approximately 27 million. Nepal is typical of many developing countries struggling to control tuberculosis. Up to 45% of Nepal’s human population is infected with TB with 44,000 active cases reported every year. Tuberculosis is also endemic in Nepal’s livestock. While livestock tuberculosis is typically caused by Mycobacterium bovis and human tuberculosis is assumed to involve primarily M. tuberculosis, people are also susceptible to M. bovis infection. There is no national livestock TB control program in Nepal, and routine screening for tuberculosis in people provided by a WHO supported DOTS program does not differentiate M. bovis from M. tuberculosis infection. Consequently, a true understanding of the dynamics of tuberculosis in people and animals is poorly understood. Tuberculosis also occurs in elephants in Nepal and may occur in other wildlife species. In Europe and the United States, cases of elephant tuberculosis most often involve M. tuberculosis, attributed to the intimate relationship between elephants and humans in captive settings. In Nepal, elephants are trained and kept for use in wildlife and forest management, tourism, and for captive breeding. A recent study revealed that the incidence of tuberculosis in captive elephants Nepal is likely greater than 15% and that both M. bovis and M. tuberculosis are likely involved. This is not surprising due to the high prevalence of M. bovis in livestock that share pastures with these elephants, but it raises new questions about the risks of this disease to elephants and other important free-ranging endangered wildlife, livestock and people that also share this environment. Tufts faculty and students are working with other U.S. and Nepalese scientists and institutions to better understand the dynamics of these two organisms, M. bovis and M. tuberculosis, in the Terai ecosystem of southern Nepal. Ongoing studies are focusing on the development of diagnostic tools that will better differentiate these two organisms in livestock, elephants and other wildlife, and people. Other research is focusing on developing effective and affordable treatment regimens for controlling tuberculosis in Nepal’s captive elephant population and in understanding the ecology of this disease to better inform policy aimed at controlling or preventing further infection.
Title:
Development of a PCR Diagnostic Technique for Differentiation of *Mycobacterium* Species in Elephant Trunk Wash Samples in Nepal

Authors:
Tierra Wilson, Donna Akiyoshi, Sujata Desai, Mahesh Bhandari, Sarad Paudel, Poornima Manandhar, Salina Manandhar, Susan Mikota, Jean Mukherjee, Gretchen Kaufman

Presented by:
Gretchen Kaufman

Departments:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine; Institute of Agriculture and Animal Sciences, Rampur, Nepal; Elephant Care International, Nepal; Central Veterinary Laboratory, Kathmandu, Nepal

Abstract:
*Mycobacterium tuberculosis* (*M. tb*) is a known threat to captive elephants in the U.S. and elephant range countries. In Nepal, it is unknown whether *M. tb* and/or *Mycobacterium bovis* (*M. bovis*) are responsible for pulmonary mycobacteriosis in elephants. *M. bovis* is endemic within Nepali cattle/water buffalo populations and *M. tb* is endemic in the human population. Captive elephants are in close association with humans and share grazing pastures with cattle, water buffalo and wild elephants providing opportunity for bidirectional transmission of both *M. tb* and *M. bovis*. Identification of the *Mycobacterium sp.* responsible for elephant mycobacteriosis is essential to understanding the mode of transmission in Nepal.

Diagnosis of elephant mycobacteriosis relies on trunk wash (TW) culture and serology. TW culture requires BL3 facilities, takes 8-12 weeks, and lacks sensitivity. Serology while sensitive, does not differentiate the various *Mycobacteria sp.* We have developed a highly sensitive gyrB-based PCR-RFLP assay capable of differentiating *M. tb*, *M. bovis*, and *M. avium*. When applied to TW samples from 22 suspected positive Nepali elephants, PCR inhibitors were localized to debris present in elephant trunks. Following inhibitor removal, PCR-RFLP was able to successfully differentiate *M. tb*, *M. bovis*, and *M. avium* in TW samples spiked with DNA but was unable to detect Mycobacteria in the 22 suspected positive elephants. Current studies are directed towards improving removal of PCR inhibitors and increasing recovery of *Mycobacterium* DNA from TW samples.
Title:
Risk Factors Associated with the Transmission of Tuberculosis in Captive Elephants in Nepal

Authors:
Lydia Scheidler and Narayan Neupane

Presented by:
Lydia Scheidler

Departments:
Cummings School of Veterinary Medicine; Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal

Abstract:
The aim of this study was to investigate the risk factors associated with the transmission of tuberculosis in captive elephants in Nepal. A 2006 study, which was the first to systematically test captive elephants in Asia for tuberculosis, found that at least 15 of the 120 elephants tested in Nepal were positive. Very little is known about the epidemiology of the disease in captive elephants in Asia. How these elephants are contracting tuberculosis and any additional risk factors, have not been documented.

We conducted a questionnaire in June and July of 2009 to identify potential risk factors for the transmission of tuberculosis in captive elephants in Nepal. The caretakers of 92 government and privately-owned elephants within and around Chitwan National Park, Chitwan District were interviewed regarding the elephants' general history, activities, diet, housing and interactions with other captive elephants, with domestic animal species, with wildlife and with people. The survey was also used to gather basic demographic data regarding captive elephants in Nepal, which has been previously undocumented. Statistical analysis to identify potential risk factors is ongoing and will be conducted with Epi Info (Center for Disease Control, Version 3.5.1).

Research into the risk factors associated with the transmission of tuberculosis in elephants has many positive implications for both human and animal health. Further knowledge on how captive elephants in Asia are contracting tuberculosis could inform the development of elephant management practices that reduce transmission, thus preventing more elephants from contracting this debilitating disease. Because tuberculosis is zoonotic, an understanding of how the disease is transmitted could also help improve the health of those in close contact with captive elephants in Asia. Increased knowledge of how tuberculosis is transmitted between people and animals also has broader public health implications for this important disease.
Title:
Characterization of HIV-1 CRF01_AE Integrase Gene in a Cohort of Vietnamese Drug Users

Authors:
Rony Barbara, Christine Wanke, Hien Duc Nguyen, Lien Trinh, Dang Duong, James Hellinger, Michael Jordan

Presented by:
Rony Barbara

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Department of Public Health and Community Medicine, Nutrition/Infection Unit, Tufts University School of Medicine; National Institute of Infectious and Tropical Diseases, Hanoi, Vietnam

Abstract:

Background: Integrase inhibitor drug-resistance mutations and polymorphisms have been characterized for HIV-1 subtype B. As integrase inhibitors (INI) are introduced into resource-limited settings, it is necessary to characterize low frequency HIV integrase drug resistant mutations (HIVDR). Low frequency INI HIVDR is characterized in a cohort of antiretroviral treatment (ART) naïve HIV CRF01_AE infected drug users from Hanoi, Vietnam.

Methods: Twelve ART naïve HIV infected individuals were randomly chosen from a cohort of 100 ART naïve individuals eligible to start ART at a large urban clinic in Hanoi, Vietnam. Standard cloning and sequencing was performed generating an average of 17 clones per specimen. Nucleotide sequences were aligned using Clustal W. All alignments were visually inspected and frameshifts were removed using MEGA 4.0. Genetic pairwise difference, measured as absolute nucleotide difference, mean pairwise difference within sequences and Neighbor-Joining (NJ) tree construction with 1,000 bootstrapping was performed using MEGA 4.0. Drug resistance was assessed using the Stanford drug resistance algorithm.

Results: Mean pairwise diversity within sequences was (range 0.95% – 1.02%); NJ tree construction demonstrated separate clustering for each patient; all sequences were CRF01_AE. PCR recombination was not observed.
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<td>INI HIVDR Mutation(s) &amp; Frequency*</td>
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<td>V165I(5/16) V72I(1/16)</td>
<td>V165I(1/18)</td>
<td>none</td>
<td>M154I(1/19) V72I(7/19)</td>
<td>V72I(1/19)</td>
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*Frequency is reported as the ratio of number of clones with mutation to the total number of clones per patient

**Conclusions:** Characterization of HIV-1 integrase in this population of HIV-1 infected ART naïve DU in Hanoi, Vietnam demonstrated the presence at low frequency of major and minor INI resistance mutations in the majority of specimens collected. Further study is warranted to assess the clinical relevance of low frequency INI- mutations in populations naïve to INIs.
Title:
Pneumonia and Influenza Hospitalization in Older Adults: The Impact of HIV Infection

Author:
Jenerius Aminawung, Siobhan Mor, Elena Naumova

Presented by:
Jenerius Aminawung

Department:
Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
During a typical flu season, influenza infection causes an average 110,000 hospitalizations and 20,000 deaths in the US. Hospitalization rates in older adults are substantially increased and this population accounts for almost 90% of influenza-related deaths. The increased morbidity and mortality in older adults is, in part, attributable to decreasing immune function with age. Persons with HIV are also particularly susceptible to negative outcomes arising from influenza infection. Several studies have described increased severity of influenza in the general HIV-positive population, but none to our knowledge have looked at the older HIV-positive population that is concurrently impacted by age-related immunosenescence. Using medical claims data from the Center for Medicare and Medicaid Services (CMS), we abstracted all records containing a diagnosis of pneumonia and influenza (P&I; ICD codes 480 - 487) in older adults (65 – 100 years) for the years 1991 to 2004. Concurrent HIV infection was identified based on the occurrence of HIV-specific ICD and DRG codes in any one of the 10 diagnostic fields. We estimated the proportion of P&I hospitalizations that occurred among HIV-positive persons, relative to the total number of P&I hospitalizations in the elderly, adjusting for year and age. Length of stay and in-hospital death were used as proxies for disease severity. Between 1991 and 2004, there were 16,381,579 P&I hospitalizations among older adults, 6,518 of which occurred among HIV-positive persons (3.98 per 10,000 hospitalizations). Concurrent HIV infection was identified based on the occurrence of HIV-specific ICD and DRG codes in any one of the 10 diagnostic fields. We estimated the proportion of P&I hospitalizations that occurred among HIV-positive persons, relative to the total number of P&I hospitalizations in the elderly, adjusting for year and age. Length of stay and in-hospital death were used as proxies for disease severity. Between 1991 and 2004, there were 16,381,579 P&I hospitalizations among older adults, 6,518 of which occurred among HIV-positive persons (3.98 per 10,000 hospitalizations). HIV-positive persons comprised an increasing proportion of the total number of P&I hospitalizations during the study period (2.73 and 5.63 per 10,000 hospitalizations). The highest proportion of HIV-positive hospitalizations was among persons aged 65 – 74 years (11.52 per 10,000 hospitalizations). HIV-positive persons hospitalized with P&I were on average younger than HIV-negative persons (70 vs. 80, p<0.001), and had longer lengths of hospital stay (14.5 vs. 11.6, p<0.001). In-hospital death occurred in 17% of HIV-positive persons compared to 12% of HIV-negative persons (p<0.001). Our preliminary results confirm that HIV-positive persons have poorer outcomes following P&I hospitalization. Future work will focus on temporal trends in mortality and potential co-morbid drivers for the discrepancy in P&I hospital outcomes between HIV-positive and -negative older adults.
Title:  
Structural Approach to Understanding the Role of the Cytoplasmic Tail of HSV-1 gB in Regulation of Fusion  

Authors:  
Jared Pitts and Ekaterina Heldwein  

Presented by:  
Jared Pitts  

Department:  
Department of Molecular Biology and Microbiology, Tufts University School of Medicine  

Abstract:  
There are four glycoproteins that are essential and sufficient for HSV-1 entry (gD, gB, and the gH/gL heterodimer). Of these, gB is the most conserved among all herpesviruses. The available structure of the ectodomain of gB reveals that gB similar to fusion proteins of other viruses suggesting that the ectodomain of gB is a key player in the fusion of the viral envelope with the host cell. In contrast, the precise role the cytoplasmic domain plays in fusion has not yet been elucidated, although it is known that the cytoplasmic domain is required for fusion. Truncations to the cytoplasmic domain of gB have been shown to either enhance or abrogate the fusion activity of gB, indicating that this domain is involved in the regulation of viral entry. For example, truncation of gB at amino acid 851 completely abolishes the fusion activity, while truncation at amino acid 868 results in a hyper-fusogenic protein as evidenced in cell-cell fusion assays.  

To better understand the molecular mechanism behind the regulatory activity of the cytoplasmic domain of gB we are pursuing the structures of the cytoplasmic domain in the wild-type, hyper-fusogenic, and fusion-null forms. Constructs have been made in which the cytoplasmic domain is connected to the ectodomain of gB via a flexible linker, removing the hydrophobic transmembrane region. These proteins have been expressed using recombinant baculovirus technology and purified by affinity chromatography. Single particle negative stain electron microscopy confirmed that constructed proteins contain both the ecto- and cytoplasmic domains. Crystals of these constructs are currently being optimized in order to acquire diffraction quality crystals. Once the structure of the cytoplasmic domain is elucidated, it will allow us to correlate structural differences among truncation mutants with their observed phenotypes and determine the mechanism in which the cytoplasmic domain participates in membrane fusion.
Title:
Syncytial Phenotype of HSV-1 gB Is Associated with Diminished Membrane Interactions

Authors:
Tirumala Chowdary and Ekaterina Heldwein

Presented by:
Tirumala Chowdary

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
In herpes viruses, glycoprotein B (gB) is a key component of the viral entry machinery, being required for membrane fusion. The cytoplasmic domain of gB is thought to negatively regulate membrane fusion by a yet unidentified mechanism. C-terminal truncations of the cytoplasmic domain of gB from Herpes Simplex virus type 1 (HSV-1) lead to either a hyperfusion or fusion-null phenotype. To provide a biochemical rationale for these phenotypes, we used circular dichroism spectroscopy and size-exclusion chromatography to characterize the secondary structure and oligomeric state of full-length and truncated cytoplasmic domains of HSV-1 gB both in solution and with lipid membranes. In solution, the full-length cytoplasmic domain is a trimer containing helical secondary structure. Moreover, the cytoplasmic domain stably associates with anionic lipid membranes and this interaction leads to increased helicity in the protein. We did not find any notable differences in secondary structure or oligomeric state of the full-length or truncated cytoplasmic domains in aqueous solution. However, the truncated and the full-length cytoplasmic domains differed significantly in terms of membrane interactions. The mutant associated with the hyperfusion phenotype, gB (801 – 868), showed a smaller increase in helical structure and a diminished association with lipid membranes. The mutant associated with the fusion-null phenotype, gB (801 – 851), showed no increase in helical structure and only minimal association with lipid membranes. Based on our results, we propose that residues 869–882 form a helix in the presence of anionic lipid membranes, which allows the cytoplasmic domain of gB to stably associate with membranes. We hypothesize that this event is an important part of the mechanism by which the cytoplasmic domain negatively regulates membrane fusion.
Title:
Fusion-Null Insertion Mutants of HSV-1 gB Adopt the Trimeric Postfusion Conformation

Authors:
Jessica Silverman, Sapna Sharma, Ekaterina Heldwein

Presented by:
Sapna Sharma

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
Glycoprotein B (gB) enables the fusion of viral and cell membranes during entry of herpesviruses. However, gB alone is known to be insufficient for membrane fusion; the gH/gL heterodimer is also required. The crystal structure of the HSV-1 gB ectodomain, gB730, has shown similarities between gB and other viral fusion proteins, leading to the hypothesis that gB is a fusogen, presumably directly involved in bringing the membranes together. In acting as a fusogen, gB must undergo dramatic conformational changes, refolding from its initial, or prefusion form, to its final, or postfusion form. The available crystal structure likely represents the postfusion form of gB; the prefusion form has not yet been determined. Previously, a panel of HSV-1 gB mutants was generated using random 5-amino-acid-linker insertion mutagenesis. Several mutants were unable to mediate cell-cell fusion despite being expressed on the cell surface. Mapping of the insertion sites onto the crystal structure of gB730 suggested that several insertions might not be accommodated in the postfusion form, and thus the insertion mutants were non-functional due to an inability to refold into the postfusion form. We have generated 5 insertion mutants as soluble ectodomains and characterized them to test whether any of them adopted a prefusion form. We show that all 5 mutants adopt a conformation that closely resembles that of the wild-type gB730. Interestingly, 1 mutant displays significant local conformational differences relative to wild-type gB. We propose that the insertions cause the fusion-null phenotype of these mutants not by preventing a postfusion-like conformation but rather by interfering with other gB functions.
Title:
High Dynamic Range and Super Resolution CMOS Image Sensors for Lens-Less Scientific Imaging Applications

Authors:
Jian Guo and Sameer Sonkusale

Presented by:
Jian Guo

Department:
Department of Civil and Environmental Engineering, Tufts University School of Engineering

Abstract:
In this poster we present our research on CMOS based image sensors with high dynamic range performance and super resolution, targeted specifically for lens-less scientific imaging. Examples of applications include cell counting, cell tracking, optical biosensing based on fluorescence detection, chemical and gas sensing, and other lab-on-chip applications. We first present a high dynamic range CMOS image sensor that utilizes a combined linear-logarithmic readout scheme to facilitate detection of low light fluorescence in presence of strong background excitation. The proposed imager adjusts each individual pixel to operate in either linear or logarithmic readout mode according to the illumination level. A direct image reconstruction using a combination of linear and logarithmic pixels forgoes the need for complicated post signal processing. For proof of concept, an image sensor chip consisting of a 16×16 pixel array and the proposed readout architecture has been fabricated in 0.5µm CMOS technology. The sensor chip has been tested and demonstrates an overall dynamic range of 121dB. During scientific imaging experiments, we have successfully imaged clusters of nano particles (CoFe₂O₄) and optical fiber sensor arrays. The CoFe₂O₄ nano particles are often used as biological tags for separation and purification of biological samples and magnetic detection, and the optical fiber sensor is a popular platform for many bio-chemical sensing applications. Aside from high dynamic range CMOS image sensors, we are also actively involved in developing super resolution image sensors with pixel size less than 0.5µm×0.5µm using advanced CMOS technologies (e.g. 65nm CMOS). Small pixel size offers tremendous advantages such as lens free imaging, optical enhancement, and super high resolution given very small imaging objects. We are working towards building a super resolution image sensor with on-chip optical-transfer-function (OTA) correction for contact cell imaging and lens-less cell counting. The target applications are low-cost, single chip medical diagnosis system for health monitoring and infectious disease screening.
Title:
Perinatal Exposure to Low Doses of Bisphenol-A Affects the Reproductive Capacity of F1 Female Mice

Authors:
Nicolas Cabaton, Perinaaz Wadia, Beverly Rubin, Maricel Maffini, Carlos Sonnenschein, Ana Soto

Presented by:
Nicolas Cabaton

Department:
Department of Anatomy and Cellular Biology, Tufts University School of Medicine

Abstract:
Bisphenol-A (BPA), a well-known endocrine disruptor used as a plasticizer and in the manufacture of polycarbonate plastics and epoxy resins has been shown to leach from food and beverage containers, dental sealants, and plastic medical devices under normal conditions of use. Exposure of pregnant rodents to low doses of BPA results in pleiotropic effects in the offspring. Many of the defects occur long after BPA exposure has ended. We have already reported alterations in body weight, mammary gland development (including the development of pre-neoplastic lesions and carcinoma \textit{in situ}), reproductive tract, hypothalamus, gonadotropin levels, estrous cyclicity and behavior in offspring exposed perinatally to BPA. We hypothesize that perinatal exposure to low doses of BPA compromises reproductive success of female offspring (F1 generation). Pregnant CD1 mice were exposed to DMSO (50%, vehicle-control), 25ng BPA/kg body weight (BW)/day (BPA 25ng), 250ng BPA/kg BW/day (BPA 250ng), 25μg BPA/kg BW/day (BPA 25μg) from the end of gestational day 8 until post-natal day (PND) 16. At delivery, F1 female pups were culled to 8 pups per litter and then weaned and housed until sexual maturity. A forced breeding protocol was used to assess overall reproductive capacity of BPA-exposed females (n=18 to 22). F1 dams were housed with non-exposed fertile males starting at 2 months of age for a period of 8 months, and the cumulative number of pups born to each mother was calculated to assess the fertility and the fecundity of this F1 generation. Mice exposed perinatally to BPA 25ng and BPA 25μg had a cumulative number of pups significantly lower compared to control starting at week 16 through week 32. Likewise, mice exposed perinatally to DES had a significantly lower cumulative number of pups compared to control, starting at week 25 through week 32. Mice exposed to 25μg BPA had significantly fewer pregnancies compared to controls. Furthermore, 40% of the female mice perinatally exposed to 25μg BPA delivered less than 4 times, and 65% had less than 6 deliveries. Our preliminary results show that the number of pups delivered by each dam is not significantly altered; however there is a tendency for BPA 25μg mice to have fewer pups after the age of 6 months. These preliminary results indicate that the reproductive outcome may be altered in mice exposed perinatally to BPA. These results highlight the impact of BPA, a contaminant of food, beverages, but also medical infusions, on fertility and reproductive aging.
Title:
Engineered Zinc-Finger Nuclease Mediated Homologous Recombination of the Human Rhodopsin Gene

Authors:
David Greenwald, Siobhan Cashman, Rajendra Kumar-Singh

Presented by:
David Greenwald

Departments:
Departments of Genetics and of Ophthalmology, Tufts University School of Medicine

Abstract:
Zinc finger nucleases (ZFNs) are chimeric proteins with significant potential for the treatment of inherited diseases. In this study, we report the design of novel ZFNs targeting the human rhodopsin gene. These ZFNs may be useful for the treatment of retinal diseases such as Retinitis Pigmentosa (RP), the most common cause of inherited blindness in the developed world. We quantitated the ability of these rhodopsin-specific ZFNs to induce a targeted double-strand break in the human genome, demonstrated their ability to induce homologous recombination of a donor DNA fragment and quantitated the frequency of ZFN-mediated homologous recombination. Relative to endogenous homologous recombination, we observed a 12-fold increase in homologous recombination and an absolute frequency of ZFN-directed homologous recombination as high as 17% in the human rhodopsin gene. Herein, we also report on several aspects of donor fragment design and in vitro conditions that facilitate ZFN-mediated homologous recombination.
Title:
Revisiting the Self-Relevance of Anti-Fat Attitudes

Authors:
Kristin Dukes, Keith Maddox, Robin Kanarek

Presented by:
Kristin Dukes

Department:
Department of Psychology, Tufts University School of Arts and Sciences

Abstract:
Previous research has revealed the pervasiveness of obesity stigma but a limited amount of this research has focused on how prejudice against overweight individuals may be related to body image and attitudes toward weight gain and loss. Contrary to conventional wisdom, Crandall (1994) suggests that prejudice against overweight individuals is not founded in self-relevant weight concerns. The current research investigated and extended this hypothesis by exploring the relationship between prejudice toward overweight individuals, self-relevant weight concerns, and body image. It was hypothesized that self-relevant weight concerns, and not prejudice, would be significantly related to measures of body image and attitudes toward weight gain and weight loss. Participants completed Crandall’s (1994) Antifat Attitudes Questionnaire (AFA) as well as questionnaires that assessed body image (body dissatisfaction and body shape concerns) and attitudes about weight gain and loss. The AFA is composed of three subscales that map onto evaluations of overweight people (Dislike), beliefs about the controllability of weight (Willpower), and self-relevant concerns about being overweight (Fear of Fat). Consistent with predictions, analyses revealed significant positive correlations between body dissatisfaction, body shape concerns, unfavorable attitudes toward weight gain, and self-relevant concerns about being overweight (Fear of Fat). Also consistent with predictions, no relationship existed between these factors evaluations of overweight individuals (Dislike) and beliefs about controllability of weight (Willpower). In line with Crandall’s previous research, these results suggest that prejudice against overweight individuals and beliefs about their ability to control weight are not based on self-relevant weight concerns. Implications for the underlying belief systems associated with body image and weight control are discussed.
Title:
Prenatal Exposure to BPA Induces Changes in Genomic DNA Methylation in Rat Mammary Gland

Authors:
Eugen Dhimolea, Tessa Murray, Michael Settles, Toshi Shioda, Perinaaz Wadia, Carlos Sonnenschein, Ana Soto

Presented by:
Eugen Dhimolea

Departments:
Department of Anatomy and Cellular Biology, Tufts University School of Medicine; Bioinformatics Core Facility, Washington State University; Massachusetts General Hospital Cancer Center

Abstract:
Prenatal exposure to environmental estrogens (xenoestrogens) may be responsible for the increased incidence of breast cancer observed in European and US populations over the last 50 years. The xenoestrogen bisphenol A (BPA) has been shown to leach from plastic food/beverage containers and dental materials. Previous studies in our laboratory reveal that fetal BPA exposure is sufficient to induce the development of preneoplastic and neoplastic lesions in the adult rat mammary gland. The mechanism underlying the origin and progression of neoplastic lesions after developmental exposure to xenoestrogens remains largely unknown. In this regard, the link between epigenetic changes and carcinogenesis is being investigated intensively. Perinatal exposure to various xenoestrogens has been shown to induce transgenerational effects via germ line DNA methylation. We hypothesized that alterations in DNA methylation could provide an explanation for the outcomes seen in adult rats exposed to BPA in utero.

To explore this hypothesis, Wistar-Furth rats were exposed to BPA (250μg/kg/day) or vehicle from gestational day 9 to birth using osmotic pumps. The animals were sacrificed at postnatal day (PND) 4, 21 and at first estrous after PND 50. Genomic DNA (gDNA) was isolated from the mammary glands and enzymatically fragmentized. The methylation-rich gDNA fragments were immunoprecipitated using a specific anti-methylcytosine antibody and subsequently hybridized to a Nimblegen ChIP array in order to determine changes in the methylation profile between BPA- and vehicle-treated animals.

A small number of gDNA regions showed persistent genomic changes at all PNDs tested: only 3 hypo-methylation and 5 hyper-methylation areas were identical at PND 4, 21 and 50 after in utero BPA exposure. Importantly, in utero BPA exposure induced hypo- or hyper-methylation in several additional regions of gDNA at PND 21 and these effects persisted at PND 50. Certain chromosomal regions including Chr1q4, Chr6q2.4, Chr7q3.4 and Chr8q3.2 were found to be especially rich in hypo- or hyper-methylated areas. We are currently identifying genes whose expression may be affected by BPA-induced changes in gDNA methylation.

Elucidation of the prenatal BPA-induced epigenetic changes may help understand the underlying developmental mechanisms that contribute to breast cancer initiation and progression in adulthood.
Title:
Feasibility Study on Using Non-Surgical Sterilization as a Means of Street Dog Population Control in Kathmandu, Nepal

Authors:
Mehnaz Aziz, Joann Lindenmayer, Gretchen Kaufman, Anette Rauch, Mahesh Bhandari

Presented by:
Mehnaz Aziz

Department:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine

Abstract:
Rabies is a major public health concern in Nepal. An average of 25,000 people receive post-exposure treatment for rabies and more than 100 people die from rabies each year. Per World Health Organization guidelines, control of the street dog population is one major component of rabies control. Both poisoning and surgical sterilization have been used in Kathmandu, Nepal in attempts to reduce the street dog population. The aim of this study was to analyze the feasibility of using an alternative population control method, non-surgical sterilization, in Kathmandu, and, in doing so, explore potential societal challenges regarding the introduction of this novel control method. A total of 60 community and 20 veterinary questionnaires were conducted to collect data on the social attitude towards the Kathmandu street dog population and its control methods. Although respondents expressed general acceptance and support for non-surgical sterilization, the study identified multiple concerns that must be addressed if a non-surgical sterilant is to be introduced in Kathmandu one day. Lack of community awareness regarding dog overpopulation and lack of government involvement in dog population management indicate the need for a government-led policy on rabies and dog population control that includes public education. The data collected in this study will facilitate future implementation of non-surgical sterilization in Kathmandu, and it may also serve as a model for potential implementation of non-surgical methods in other developing countries.
Title:
Assessing the Effectiveness of the Dog Sterilization and Rabies Control Program at IAAS, Rampur, Nepal

Authors:
Colin Basler, Indra Shah, Subir Singh, Gretchen Kaufman

Presented by:
Colin Basler

Department:
Department of Environmental and Population Health, Cummings School of Veterinary Medicine

Abstract:
Rabies continues to pose a serious threat in many parts of the developing world, including Nepal. In the recent past, a dog sterilization and vaccination program has been implemented in the Chitwan district of Nepal in order to reduce the incidence of rabies and increase the capacity to implement rabies prevention programs within the country. In order to determine the effectiveness of the program, 120 residents of four communities where the sterilization/vaccination program has been operating were surveyed and their responses were compared to 120 residents in four similar communities which the program has not reached. A cross-sectional cluster sample survey was used in order to determine the community members’ knowledge of rabies as well as their attitudes about the rabies control program and their willingness to use the services the program provides. It was determined that overall, the rabies control program has had a positive impact but that there are some specific areas for improvement.
Title:
Vitamin D Status of Elderly Mayan Residents of the Western Highlands of Guatemala

Authors:
Sohil Sud, Gabriela Montenegro-Bethancourt, Robert Heaney, Laura Armas, Noel Solomons, Odilia Bermudez

Presented by:
Sohil Sud

Departments:
Department of Public Health and Community Medicine, Tufts University School of Medicine; Center for the Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM), Guatemala; Osteoporosis Research Center, Creighton University

Abstract:
Introduction: While the prevalence of various chronic diseases is known to be high among elderly Guatemalan Mayans, and research linking vitamin D (VitD) deficiency to such conditions continues to grow, little is known about Mayan levels of vitamin D. Geographic factors (e.g., residence in areas receiving ample sunlight with little air pollution at high altitudes and latitudes near the equator) favor conjectures that they may have optimum VitD levels, but demographic factors (e.g., darker skin pigmentation and older age) support speculations of low 25(OH)D status.

Objectives: To obtain descriptive information about 25(OH)D levels in elderly Mayans, and to compare levels obtained between urban and rural subjects.

Methods: Blood samples were collected from 108 healthy elderly Mayans (mean age 69y) from urban (n=84, 50% male) and rural (n=24, 50% male) areas of Quetzaltenango, Guatemala during summer 2008. Samples were transported to the USA, where serum 25(OH)D levels were determined by radioimmunoassay in a DEQAS-certified laboratory.

Results: Mean serum 25(OH)D levels were 53.3±15.0 nmol/L of the total sample, and decreasing 25(OH)D levels correlated with increasing age (r=-0.28, p=0.004), 3.7% (n=4) of the study population maintained optimal levels of 25(OH)D (>80 nmol/L), 50% (n=54) had suboptimal levels (50-80 nmol/L), and 46.3% (n=50) were considered vitamin D-deficient (<50 nmol/L). Males had significantly higher levels of VitD (58.2±16.5 nmol/L) than female subjects (48.4±11.6 nmol/L, p=0.001), and urban subjects had non-significantly higher levels (55.0±15.3 nmol/L) than rural subjects (47.4±12.4 nmol/L, p=0.228).

Discussion: Despite residing in an optimal geographic location to receive adequate sunlight exposure, the vast majority of elderly Guatemalan Mayans in Quetzaltenango have suboptimal or deficient levels of VitD. This data, along with results indicating that urban dwellers may have higher levels of 25(OH)D than rural dwellers, introduces the possibility that sunlight exposure may play less of a role in achieving optimal VitD status than
previously expected in this population. Although larger surveillance studies and investigations into the diet and outdoor activities of elderly Mayans are necessary, this pilot investigation indicates that VitD supplementation may prove beneficial to this population.
Title:
Frequency of R267S and A379V Polymorphisms Within the β-carotene 15,15’ Monoxygenase Gene in a Mayan Population

Authors:
Georg Lietz, Gabriela Montenegro-Bethancourt, Sohil Sud, Klaus Schueman, Noel Solomons

Presented by:
Sohil Sud

Departments:
Department of Public Health and Community Medicine, Tufts University School of Medicine; School of Agriculture, Newcastle University, United Kingdom; Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala; Science Center Weihenstephan, Technische Universität, München, Germany

Abstract:

Background: Two common single nucleotide polymorphisms (SNPs)—R267S and A379V—in the β-carotene 15,15’-monoxygenase (BCMO1) gene are largely responsible for the variable efficiency of β-carotene conversion into retinal. The distribution of these SNPs has been described in European, Asian, and African ethnicities, but till date not in indigenous Latin American populations. We analyzed DNA from a Guatemalan Mayan population to determine the genotype and allelic frequencies of R267S and A379V, and compared it to other distinct ethnic groups.

Methods: Saliva samples were obtained from n=119 Mayan schoolchildren (n=60 males; mean age 12.1y +/- 1.7) in urban Quetzaltenango, a highland province of Guatemala. Specimens were shipped to UK for analysis, where DNA was extracted and purified from n=117 samples. BCMO1 polymorphisms were analyzed using mass spectrometry, and the distribution of R267S and A379V SNPs in the Mayan population was determined.

Results: Allele frequencies of R267S and A379V were 17% and 19%, respectively, among Mayan schoolchildren. Genotype frequency distributions for both SNPs were similar in the Mayan, Han Chinese and Japanese groups, and significantly lower than distributions found among European ancestry groups. The Mayan group also showed a combined 267S+379V variant allele frequency of 5.5%, which was similar to Han Chinese and Japanese groups, and lower than Europeans descendents.

Discussion: The low prevalence of R267S and A379V SNPs in Guatemalan Mayan schoolchildren signifies that they may convert β-carotene into retinal more efficiently than their European counterparts. This finding corroborates the prevailing pattern of dietary vitamin A supply from plant sources in Guatemalan society, for which highly effective cleavage of β-carotene is prerequisite. Similar SNP distributions have also been found in Asian ethnic groups. Whether this parallel is due to a common ascent of present-day Mayans and Asians, or if both populations developed the same response to a common dietary problem cannot be decided on this database.
Title: Evaluation of Water, Sanitation and Hygiene Practices of Kuna Indians Living in Arraiján, Panamá

Author: Rodela Khan

Presented by: Rodela Khan

Department: Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:

Introduction: The primary aim of this project was to obtain baseline information on existing water and sanitation facilities and hygiene practices of the Kuna Indian population living in Boo Yala in the district of Arraiján, Panamá. Kuna are the second largest indigenous group in Panamá. Indigenous groups face problems such as limited access to healthcare, education, poor living conditions and significant health effects like malnutrition.

Methods: This project was conducted with the Panamá West Regional branch, under the Ministry of Health of Panamá. A structured questionnaire with observation was completed in 30 households. Two in-depth, key informant interviews were also conducted. Additionally, photography was used to document physical conditions in the community. Using information collected during the interviews, basic demographic data like household income and size were calculated. Major themes and concepts were developed after content analysis and coding of interviews, structured observations and personal notes.

Results: The monthly income for the majority of families in this sample fell within $201 – $300 US dollars below the national average in Panama among non-indigenous workers of $322. The average number of household members was 7.1, while the national non-indigenous average is four people. The primary potable water source for Boo Yala is a piped water system that was built in 2006 by a local non-profit organization, which every household has access to. Nearly three quarters of homes in Boo Yala were classified as using pit latrines in poor condition; less than a quarter were classified as adequate and only one home was characterized as being in good condition. Garbage disposal emerged as a major issue and since garbage collection is unreliable and inaccessible to many, burning and burying are the preferred methods of waste disposal.

Conclusion: Latrine structures must be improved along with addressing garbage disposal methods with alternative methods like community composting. In order for improvements specifically in sanitation and health, much more progress needs to be made by increasing the physical infrastructure and accessibility to this community.
Title:
Impact Assessment of Community Health Agents in the Somali Region of Ethiopia and Gender Disparities in Access to Primary Healthcare

Authors:
Andrew Catley, Gezu Bekele, Alison Napier

Presented by:
Andrew Catley

Departments:
Feinstein International Center, Gerald J. and Dorthy R. Friedman School of Nutrition Science and Policy; Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
The Somali region in eastern Ethiopia is one of the least developed areas of the world. Covering around 400,000 square kilometers, the region is characterized by insecurity, harsh physical environment, and livelihoods based on mobile pastoralism. Crude life expectancy for men has been estimated at 41 years and for women 33 years. Between 2002 and 2007 Save the Children US (SC US) implemented a health program in selected districts of the Somali region, and this program included support to community health agents (CHAs). Tufts University conducted an impact assessment in 2008, focusing on the activities of CHAs and examining the use of these workers by women and men. The impact assessment used five indicators of service provision viz. accessibility, availability, affordability, acceptance and quality, and asked both women (n=200) and men (n=200), sampled randomly, to score these indicators for the different health service providers/facilities which they used. A standardized participatory method called matrix scoring was used, and supported with semi-structured interviews to elicit explanations for the scores offered.

The types of health service providers/facilities used by women were traditional birth attendants, CHAs, government health centers and ‘other’ service providers comprising traditional healers and informal drug sellers; men used CHAs, government health centers, a hospital and ‘other’ service providers. CHAs received relatively high scores from both women and men for all service indicators apart from quality, but were also significantly more accessible, available, affordable and acceptable to women compared with men. This result was explained by men’s preference for health clinics over CHAs, and their ability to travel to and afford health clinic services relative to women. These results reflected cultural discrimination against women in Somali pastoralist communities, as reported in other studies. The main area for improving the CHA system was to improve quality, and this required the CHAs to be allowed to provide a wider range of clinical services, with related training, supervisory and policy support. Such improvements were likely to provide particular health benefits for women.
Title:
Health Care Access Among Kuna Indians Living in the District of Arraiján, Panamá

Author:
Keith Lividini

Presented by:
Keith Lividini

Department:
Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:

**Background:** Many Kuna Indians of the Comarca Kuna Yala in Panamá have left in search of education and employment and have established the community of Boo Yala in the district of Arraiján. Health officials working with the Panamá West Region of the Ministry of Health have documented many health problems among the Kunas living in Boo Yala, including malnutrition and diarrhea among others. Health officials have also noticed limited health care use within Boo Yala.

**Purpose/Objectives:** The objective of this study was to gather information about: 1) the type of health services utilized by the people of Boo Yala; 2) the accessibility of health services; and 3) the quality of health care received in those facilities.

**Methods:** A survey containing questions about health care use, accessibility, and quality of care was developed with nurses at the Ministry of Health, Panamá West Region. The questionnaire was translated, edited, and checked for cultural accuracy with the nurses who had worked in Boo Yala. The questionnaire was administered by interview to 50 households in Boo Yala with the help of a nurse from the Panamá West Region and a community leader in Boo Yala. All data analysis was done using EpiInfo™.

**Results:** The survey accounted for 50 homes and 351 people of Boo Yala. The average number of persons per household in this sample was 7.0 and the median age of the people of Boo Yala was 22 years. About 43% of households had an average monthly family income of between $200 - $300. Greater than 40% of people accessed no form of health care either for sickness or prevention. The type of health care used most frequently was the polyclinic which was accessed primarily by the public bus system. No community health center exists near Boo Yala. For over 70% of the people in the sample, transportation time to the polyclinic is greater than 1 hour using the public bus system and requires multiple transfers. Waiting time within the polyclinic was reported to be an additional 1-2 hours by greater than 50% of those surveyed. In contrast, most of the Kunas in Boo Yala were satisfied with the care they received, felt that their needs were adequately met when care was accessed, and that there were no barriers due to language and cultural differences, or discrimination.

**Conclusions:** The primary barriers to health care access for the people in Boo Yala are distance from the health care facility and time waiting for care. There do not appear to be significant barriers due to cultural differences or discrimination. The community of Boo Yala needs a community health center (centro de salud) and a more direct route of transportation to the clinics.
Title:
Improving Oral Health in Haiti: Preliminary Assessment Strategies for Implementing Guidelines for an International Salt Fluoridation Project

Authors:
Aidee Herman, Melvin Miller, Jeffrey Griffiths, Matthew Navidomskis, Todd Walker, Samantha Jordan, Nicole Muller-Cesar

Presented by:
Aidee Herman and Todd Walker

Departments:
Department of Periodontology, Tufts University School of Dental Medicine; Department of Public Health and Community Medicine, Tufts University School of Medicine; Massachusetts Hispanic Dental Association; Institute of Human and Community Development, Haiti

Abstract:
The program objective is to vigorously evaluate strategies to implement international salt fluoridation program guidelines in Haiti. Oral disease is a critical element in overall health status in Central American, Latin American, and Caribbean regions. In Haiti, the majority of the population resides in rural areas without access to community fluoridated water. In particular, dental caries remain at a high prevalence and severity. Additionally, the prevalence and severity of dental caries is greater in low-income and poorly-educated population segments.

While 65.8% of the U.S. population has fluoridated water, a sizeable number (34.2 %) of U.S. residents still do not have access to fluoride in their water. Internationally, these levels fluctuate for a variety of reasons including economic hardship and geographical barriers.

Salt fluoridation is as effective as water fluoridation, and in most instances, less costly. The addition of micro-nutrients (e.g. fluoride, iodine, Vitamin A) to the diet to prevent disease incurs inherent cost. However, fluoridated salt is generally no more expensive than non-fluoridated salt. The Pan-American Health Organization (PAHO) currently has mature policies, adequate infrastructure, and sustainable fluoridation programs in various countries, excluding Haiti.

A feasibility assessment using existing fluoridation data will be performed and subsequently utilized to implement a program as follows: (a) Coordinate a comprehensive network of project partners (domestically and abroad) to further the goal of ‘global oral health’; (b) Identify and evaluate specific barriers; (c) Policy analysis; and (d) “Salt Fluoridation Health Campaign” in collaboration with the University of Haiti and the Institute of Human and Community Development.
Title:
Under-5 Mortality and Birth Order in Cambodia: A Cross-Sectional Analysis of Data from the Cambodia Demographic and Health Survey (CDHS) 2005

Author:
Kimberly Petko

Presented by:
Kimberly Petko

Department:
Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
Cambodia has the highest rates of under-5 mortality in the region despite global efforts to reduce mortality. A cross-sectional study of data from the Cambodia Demographic and Health Survey (CDHS) 2005 was conducted to examine the relationship between birth order number and under-5 mortality to determine 1) if there is a significant correlation between mortality and birth order and 2) if mortality rates significantly differ by birth order. Births that occurred during 2001-2005 were studied. The sample included 8180 cases, and a total of 597 children died during the five year study period. Sample characteristics were determined through descriptive statistics. Mortality rates were calculated for under-5 mortality, and its two subcategories: infant and child mortality. Mortality and mortality rates were analyzed across birth order using a cross-tabulation, Spearman’s correlation test, and a binary logistic regression, which included the covariates: maternal education, wealth, residence, sex of child, and multiple births. The results show a significant positive correlation between under-5 mortality and birth order. As birth order increases, mortality rates were found to significantly increase. Also, the majority of under-5 deaths occur during infancy. This evidence suggests that family planning interventions should aim to reduce the number of extreme birth order pregnancies as well as provide increased support for mothers and infants to reduce mortality within the first year of life.
Title:
Creation of an Open Database of Virtual Patients for Educational Use Demonstrating the Most Common Diseases Effecting Global Health

Authors:
Stanley Jacobson, Scott Epstein, Jeffrey Griffiths, Joseph Polak, Susan Albright

Presented by:
Stanley Jacobson

Departments:
Departments of Anatomy and Cellular Biology and of Public Health and Community Medicine, Tufts University School of Medicine; Office of Educational Affairs, Tufts University; Technology for Learning in the Health Sciences, Tufts University; Department of Radiology, Tufts Medical Center

Abstract:
We believe the development of a database of virtual patients from CT images of living patients and cadavers forms a realistic and predictive model of global health and disease. In this presentation, we will present virtual patients that demonstrate the most common causes of death. A virtual patient (VP) is developed in a case history format and is defined as: “an interactive computer simulation of real-life clinical scenarios for the purpose of medical training, education, or assessment. These VP’s will be developed from cadavers and living patients using cutting-edge medical imaging and computer manipulation to demonstrate the effects of the most common diseases leading to death (in the US National Vital Statistics of the CDC for 2007). These diseases include: Coronary heart disease (31%), Cancers (26%), Stroke and cerebrovascular diseases (7.5%), Chronic respiratory diseases/COPD (7.1%), Accidents (4%), Diabetes (4%), Alzheimer’s disease (3.8 %), Influenza and Pneumonia (3.1%), Nephritis (2.8 %) and Septicemia (2.7%). Outside the US, the World Health Organization (WHO) shows similar world-wide finding in the more affluent countries but in other areas of the world Diarrheal diseases, HIV/AIDS, Malaria, and Tuberculosis are on the rise and with most diseases now truly international and as we do not commonly in the United States see patients with Diarrheal disease, HIV/AIDS, Malaria or TB we have formed a collaboration with Anatomy Departments in East Africa, University of Nairobi, Kenya; Muhimbili University College of Health Sciences in Tanzania, and Makerer University in Uganda where they see many patients with these diseases. The Christian Medical College in Vellore India, has also expressed interest in participating in this project. Our method at each school is a team approach combining faculties from the Basic Sciences (Anatomy) and Clinical Sciences (Medicine, Neurology Radiology, Pathology and Surgery) to reach our goal of a VP. For each case suitable, self-quiz questions related to the disease seen in the patient, and their answers will be provided. Internet links with appropriate cross references to these findings would be provided to reliable open sources including sites such as the MedLinePlus.gov, Answers.com, and MayoClinic.com databases. The CT image stack from each patient would then be subjected to a 3D volumetric
reconstruction using the OsiriX program, along with the flash program to make it more interactive. When completed, each VP would be placed on an open website, such as the Tufts OpenCourseWare site. The TUSK staff have built a tool to extract a case into an open source player to be accessible to anyone anywhere. This unique and comprehensive compilation of human diseases developed as VP’s would be readily available for all to share via the Internet and readily available to rural or underserved populations, and academic patient care environments, thus forming an important resource for medical education and public health and also reduce health care costs by sharing educational tools across health care institutions that will improve training and the quality of patient care permitting the broadest dissemination of these most significant public health issues. Over the course of last semester, 14 virtual patients were created incorporating the CT images and the 3D reconstructions on diseases ranging from Arthritis to asbestosis. Student evaluation of these cases will be shared.
Title:
Progress in Neglected Disease Drug Development

Authors:
Joshua Cohen and Andrew Wilson

Presented by:
Joshua Cohen

Department:
Tufts Center for the Study of Drug Development

Abstract:

Background: In a Lancet article published in 2002, Trouiller et al. wrote a call to action on neglected disease drug development, suggesting inadequate funding was responsible for relatively few new approvals (16) targeting neglected diseases between 1975 and 1999. Since that publication, significantly more resources have been allocated to the development of drugs targeting neglected diseases. This paper reassesses the numbers in Trouiller et al., evaluates progress in neglected disease drug development since 2000, and explains how increased numbers of approvals are a necessary but insufficient condition to improving access.

Methods/Principal Findings: To assess numbers of approvals targeting neglected diseases, we identified which diseases are considered “neglected.” Here, the G-Finder report served as a benchmark. Using PharmaProjects and IMS R&D Focus databases as well as websites from a wide range of drug regulatory agencies, we calculated numbers of new drug approvals and indications. Also, we examined the World Health Organization’s Essential Drug List (EDL) to see which drugs and indications had been placed on the list. Upon recount we found that between 1975 and 1999 more drugs (36) targeting tropical diseases and tuberculosis were approved than reported in Trouiller et al. (16). Overall, we found 59 drug approvals between 1975 and 1999 targeting G-Finder neglected diseases. The WHO included 85% of these drugs on the EDL. However, in the period 2000-2008 period, despite greater funding, only 21 drugs and vaccines for neglected diseases were marketed. Of these, WHO placed 38% on the EDL.

Conclusions/Significance: There has been uneven progress in neglected disease drug approvals, with malaria appearing to benefit most in the short run from increased funding, while less success has been booked in other disease categories. Uneven progress suggests funding should be better targeted, particularly with regard to neglected diseases that have hitherto received scant attention. In addition, policymakers should focus on the larger question of access. Besides drug development, there are the issues of EDL listing, availability, affordability, and adoption.
Title:
Multiplexed Sensing Based on Brownian Relaxation of Magnetic Nanoparticles Using a Compact AC Susceptometer

Authors:
Kyoungchul Park, Sameer Sonkusale, Tim Harrah

Presented by:
Kyoungchul Park

Departments:
Department of Electrical and Computer Engineering, Tufts University School of Engineering; Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
A novel multiplexed sensing scheme based on the measurement of the magnetic susceptibility of the affinity captured target molecules on magnetic nanoparticles in liquid suspension is presented. The AC magnetic susceptibility measures Brownian relaxation behavior of biomolecules bound to magnetic nanoparticles (MNPs) that are related to its hydrodynamic size. We have designed and developed a room temperature, compact AC susceptometer to measure complex AC magnetic susceptibility of such magnetic nanoparticles. Our AC susceptometer exhibits high sensitivity in magnetic fields as low as 0.1 Gauss for 1 mg/ml concentration and 5 ul volume, and is fully software programmable. The capability of biological sensing using the proposed scheme has been demonstrated in proof of principle using the binding of biotinylated horseradish peroxidase (HRP) to streptavidin coated MNPs. The proposed technique and instrument are readily compatible with lab-on-chip applications for point of care medical applications. The proposed sensing paradigm based on brownian relaxation of MNPs clearly offers plenty of opportunities for biological and medical applications such as detection of virus, DNA protein or cancer cells.
Title:
Large Scale Identification of *Wolbachia pipientis* wMel Type IV Effectors

Authors:
Irene Newton, Cammie Lesser, Ralph R. Isberg

Presented by:
Irene Newton

Departments:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine; Massachusetts General Hospital; Harvard University

Abstract:
*Wolbachia pipientis* is an obligately intracellular endosymbiont found in a diverse array of insects and nematodes. Estimates suggest that over 15% of insect species harbor the parasite and in insects, they lead to reproductive manipulations including cytoplasmic incompatibility, male killing, parthenogenesis, and feminization (Werren review, 2008). Regardless of the effect on the host, all invading intracellular microbes face similar challenges: adherence to host, induction of phagocytosis, escaping lysosome fusion, and subsequent proliferation. We know from genetic and genomic studies that both mutualists and pathogens use similar strategies to overcome these challenges, including the use of secretion systems. *Wolbachia pipientis* wMel encodes a type IV secretion system that directly injects into host cells proteins that manipulate eukaryotic cellular processes. Identification of these translocated proteins would allow us to better understand how *Wolbachia* promotes infection and causes reproductive effects. Here we present a large-scale functional genomic screen of putative *Wolbachia* translocated substrates using yeast as a model system. Substrates of bacterial protein translocation systems can often confer toxic phenotypes when expressed in the yeast *Saccharomyces cerevisiae* (Heidtman et al., 2008; Slagowski et al., 2008; Kramer et al., 2007). Identification of this subset of effectors only requires that the bacterial orfs be expressed in yeast, making this approach perfect for the study of *Wolbachia*, which cannot be cultured or genetically manipulated. Over 100 putative translocated substrates were identified in the wMel genome using bioinformatics methods and of these, 60 have been targeted for expression in yeast. We discuss the diversity of domains found in this effector pool and the initial phenotypic characterization of members that are expressed in yeast.
Title:
Large Arrays of Functionalized Carbon Nanotubes on Silicon CMOS for (Bio)chemical Sensing Applications

Authors:
Sam MacNaughton, Chia-Ling Chen, Mehmet Dokmeci

Presented by:
Sam MacNaughton

Departments:
Department of Electrical and Computer Engineering, Tufts University School of Engineering; Department of Electrical and Computer Engineering, Northeastern University

Abstract:
Single chip nano- and micro-sensor arrays are highly desirable as technologies for global health and as biochemical sensors for detection of infectious diseases. Towards that goal, we present a versatile carbon nanotube based (bio)chemical sensing platform integrated onto complementary metal oxide semiconductor (CMOS). Highly dense networks of single walled carbon nanotubes (CNTs) functionalized with and without single stranded DNA (ss-DNA) were assembled onto CMOS circuitry using low voltage dielectrophoresis. Large arrays of cross-reactive CNT based nanochemical sensors on CMOS benefit from high throughput readout and signal processing using on-chip circuitry. Exotic electro-optical transport properties, and superior biocompatibility makes CNT-based sensor arrays as an ideal platform for routine analysis of blood, saliva, urine for point-of-care medical diagnostics. We present results on integrate CMOS-CNT microsystems for physical, environmental, and chemical sensing with superior performance.
Title:
The Mechanisms of the $\alpha_3\beta_1$ Integrin-Mediated Inflammatory Response to B. burgdorferi

Authors:
Meghan Lavalley Marré and Linden Hu

Presented by:
Meghan Lavalley Marré

Departments:
Department of Immunology, Tufts University School of Medicine; Division of Geographic Medicine and Infectious Disease, Tufts Medical Center

Abstract:
Recognition of borrelial products by toll like receptors (TLRs) is thought to play an important role in both the development of inflammation as well as control of infection. However, mice deficient in TLR2 or the TLR adaptor protein MyD88 do not exhibit reduced inflammation during infection, suggesting a role for additional host receptors in the initiation of the inflammatory response. Previous work in our lab has identified $\alpha_3\beta_1$ integrin as an additional host receptor for B. burgdorferi in both non-phagocytic chondrocyte and phagocytic macrophage cell cultures. Because inhibition of $\alpha_3\beta_1$ integrin or TLR2 signaling results in a greater than 70% decrease in B. burgdorferi-induced cytokine expression, we hypothesize that $\alpha_3\beta_1$ integrin cooperates with the TLR2/MyD88 signaling pathway to regulate the activation of the host inflammatory response. Indeed, work in other systems suggests that there may be cross-talk between TLR and integrin mediated signaling. We are therefore exploring the mechanisms by which TLR and $\alpha_3\beta_1$ integrin signaling contribute to inflammation in response to B. burgdorferi and subsequent control of infection. If selected to participate in the Research Day on Global Health and Infectious Disease, we would present our on-going work defining the signaling pathways downstream of TLR2 and $\alpha_3\beta_1$ integrin in different cell types and discuss evidence for how these signaling pathways intersect and potentially cooperate to shape the host immune response.
Title:
Serum Micronutrient Status of HIV-Positive and HIV-Negative Drug Users in Buenos Aires, Argentina

Authors:
Heidi Sheehan, Jorge Benetucci, Estela Muzzio, Liliana Redini, Jorge Naveira, Marcela Segura, Mercedes Weissenbacher, Alice Tang

Presented by:
Heidi Sheehan

Departments:
Department of Public Health and Community Medicine, Tufts University School of Medicine; University of Buenos Aires, School of Medicine, Buenos Aires, Argentina

Abstract:
Background: Low serum micronutrient levels and malnutrition are associated with increased HIV transmission, HIV disease progression and mortality. Drug abuse may exacerbate food insecurity and may create more oxidative stress leading to increased risk.

Methods: We enrolled 205 former and current drug users (12% women, 34% HIV positive) into a cross-sectional study in Argentina. Each subject completed a demographic and food security questionnaire, medical history, 24-hour dietary recall and fasting blood draw. We measured serum carotenoids, retinol, selenium and α-tocopherol. Using multiple linear regression we examined correlates of serum micronutrients, including HIV, Hepatitis C, gender, age, BMI, recruitment site, vitamin supplements, cholesterol, injection and non-injection drug use, and alcohol use.

Results: The majority of participants (82%) had low selenium levels (<85ug/l) and a minority (10%) had low α-tocopherol levels (<500ug/dl), regardless of HIV status. Low retinol (<30ug/dl) and low total carotenoid levels (<29.5ug/dl) were more prevalent in HIV-positive drug users than HIV-negatives (retinol: 45% vs. 10%, p <0.001; carotenoids: 9% vs. 1%, p=0.01). A self-reported 5-item index showed an overall 38% prevalence of food insecurity. In our final regression models of serum micronutrients, we found HIV/HCV co-infection was associated with lower carotenoids (-16.6ug/dl, p=0.03), selenium (-24.1ug/l, p=0.002), and retinol (-7.6ug/dl, p=0.0008) compared to HIV-/HCV- reference group. Subjects with HIV alone, on average had lower carotenoids (-30.3ug/dl, p=0.0002) and selenium (-26.5ug/l, p=0.0003) than the reference group. Having HCV alone was associated with lower selenium (-23.8ug/l, p=0.005) and α-tocopherol (-101.5ug/dl, p=0.02).

Conclusions: Our results indicate some of the first evidence of high rates of selenium and retinol deficiencies in South American current and former drug abusers. Food and vitamin supplement interventions may be warranted in drug abusers who are at high risk of transmitting HIV and hepatitis. The association of lower micronutrient levels with HIV infection suggests that the HIV population may particularly benefit from intervention.
Title:
Nutrition, Metabolism, and HIV Research in International Populations of Drug Users

Author:
Alice Tang

Presented by:
Alice Tang

Department:
Department of Public Health and Community Medicine, Tufts University School of Medicine

Abstract:
HIV-positive and HIV-negative drug users are at risk of nutritional compromise, resulting from poor dietary quality, food insecurity, malabsorption, physical activity (or inactivity), co-morbidities, and direct metabolic effects of specific drugs of abuse. Drug users may be at risk of both under-nutrition and over-nutrition.

The Tufts Nutrition Collaborative-Center for Drug Abuse and AIDS Research (TNC-CDAAR), is one of the first CDAAR programs in the country. TNC-CDAAR represents a partnership between 3 East Coast medical institutions and their affiliated hospitals: Tufts University, Boston, MA; Brown University, Providence, RI; and Johns Hopkins Bloomberg School of Public Health, Baltimore, MD. Each institution has distinct research emphases in HIV-infection, drug addiction, and nutrition that offer great potential for research collaboration and synergy. The long-term vision of the TNC-CDAAR is to generate research on nutrition and metabolic disorders among drug users at risk for or with HIV-infection, and become a resource center for other institutions and investigators who want to conduct similar research.

We are currently forging research connections around the world, in populations of drug users in countries such as Argentina, India, and Vietnam. We aim to determine and compare prevalence, incidence and risk factors of malnutrition and other nutrition and metabolic abnormalities such as HIV-related lipodystrophy, cardiovascular disease, bone loss, and liver disease among these geographically diverse populations. For instance, we have found a substantially higher prevalence of low serum selenium and Vitamin A levels in drug users in Argentina. Total energy intake appears to be substantially lower among HIV-negative drug users in Vietnam compared to HIV-positive drug users in Vietnam and drug users in the other countries. In addition, patterns of dietary intake and food insecurity differ across sites. These types of results will help us to develop specific and targeted interventions within each country, as well as help us to develop a more global understanding of the competing and contributing risk factors for HIV-related nutrition/metabolic abnormalities in drug users.
Title:
Resistance of *C. difficile* to Cationic Antimicrobial Peptides

Authors:
Shonna McBride and Abraham L. Sonenshein

Presented by:
Shonna McBride

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
*Clostridium difficile* causes a potentially fatal intestinal disease that is increasing in incidence and severity. These infections are often chronic and incredibly difficult to eradicate. Though this organism presents an enormous public health burden, little is understood about how *C. difficile* colonizes the human intestine. In order to persist in the intestinal environment, the bacteria must cope with a continuous onslaught by host defenses. Cationic antimicrobial peptides, or CAMPs, are small positively charged molecules that have microbicidal activities. Humans produce a variety of CAMPs that are concentrated in areas of the body that routinely encounter microorganisms, such as mucous membranes, skin, and the intestines. These peptides play a critical role in innate host defenses, preventing the growth and spread of both gram-positive and gram-negative bacteria. Naturally, many organisms have evolved mechanisms to circumvent the killing effects of CAMPs. Using model CAMPs, we have established that *C. difficile* is not only sensitive to these compounds, but also responds to low levels of CAMPs by expressing genes that lead to CAMP resistance. Using Real-Time PCR analysis, we found that genes responsible for D-alanine substitutions to the cell wall (dlt operon) are induced in the presence of the CAMP, Nisin. We determined that insertional disruption of the first gene in this cluster, dltD, leads to increased sensitivity to multiple CAMPs. These results provide the first evidence of a *C. difficile* gene associated with antimicrobial peptide resistance. Furthermore, investigating mechanisms of antimicrobial peptide resistance presents an additional direction for exploring treatments of *C. difficile* infections.
Title:
A Leap Toward the Identification of Alleles Conferring Resistance to the Protozoan Parasite Babesia microti

Authors:
Jamie Fierce, Jennie Chan, Carrie Wilson, Sam Telford, Jeffrey Gelfand, Henry H. Wortis, Edouard Vannier

Presented by:
Edouard Vannier

Departments:
Division of Geographic Medicine and Infectious Disease, Tufts Medical Center; Department of Pathology, Tufts University School of Medicine; Department of Environmental and Population Health, Cummings School of Veterinary Medicine; Massachusetts General Hospital

Abstract:
Not available.
Title:
Inhibiting Germination by Spores of the “Superbug” Clostridium difficile

Authors:
Joseph Sorg and Abraham L. Sonenshein

Presented by:
Laurent Bouillaut

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
Infections caused by Clostridium difficile are typically associated with antibiotic use. Treatments of C. difficile-associated diseases, which can range from severe diarrhea to pseudomembranous colitis, cost the United States healthcare system $3.2 billion annually. C. difficile is a spore-forming, anaerobic bacterium that is naturally resistant to many different antibiotics. Spore formation by C. difficile is a significant obstacle to overcoming hospital-acquired C. difficile-associated diseases. An environment contaminated with C. difficile spores is difficult to clean because the dormant spores are resistant to heat, radiation, chemicals and antibiotics. To cause disease, however, spores must germinate and grow out as vegetative cells. We have shown that C. difficile spores sense taurocholate, a bile salt, and glycine as co-germinants. Chenodeoxycholate (CDCA), a bile salt that is structurally similar to cholate, does not stimulate C. difficile spore germination but inhibits bacterial growth. We now show that CDCA competitively inhibits taurocholate mediated germination. These results suggest C. difficile spores sense a changing salt spectrum so they do not germinate in the aerobic small intestinal environment, an environment that would not support bacterial growth.
Title:
Creating a Mutant Library Using Mariner-Based Random Mutagenesis in \textit{Clostridium difficile}

Authors:
Laurent Bouillaut and Abraham L. Sonenshein

Presented by:
Laurent Bouillaut

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
Because of limited genetic tools for \textit{C. difficile} manipulation, our ability to understand the molecular mechanisms controlling sporulation and pathogenesis of \textit{C. difficile} has been hindered. We have developed a new genetic approach allowing random inactivation of \textit{C. difficile} genes based on the extensively used Mariner eukaryotic transposition system. The Mariner system is composed of a DNA fragment that encodes a transposase and two inverted terminal repeat regions (ITRs) necessary for mobility. In our approach, we used the transposon Tn916 to introduce the mobile element of the Mariner into the \textit{C. difficile} chromosome. This mobile element is composed of an antibiotic gene flanked by the two ITRs and has been inserted into Tn916 by recombination with the tetracycline gene. The modified transposon was then transfer to \textit{C. difficile} by conjugation. Next, we introduced the Mariner transposase under the control of different promoters (Pgdh or PtcdR, glutamate dehydrogenase or toxin gene regulator promoter, respectively) on an \textit{E. coli}–\textit{C. perfringens} shuttle vector which is known to be unstable in \textit{C. difficile}. Instability of the plasmid is essential to create stable insertions in the \textit{C. difficile} genome, since the loss of the Mariner transposase locks the mobile element at a specific site.

Experiments are ongoing to determine the frequency of transposition and to generate a mutant library that could be used to identify genes that are required for important aspects of \textit{C. difficile} physiology, virulence and sporulation.
Title:
The Effect of Calcium Supplementation on Periodontal Disease in a Salivary Hypofunction Population

Authors:
Medha Singh and Athena Papas

Presented by:
Medha Singh

Department:
Division of Public Health Research and Oral Medicine, Tufts University School of Dental Medicine

Abstract:

Background: Tooth loss and systemic osteoporosis affects large numbers of elderly men and women. Animal and human studies of calcium intake, bone mineral density and tooth loss suggest that low dietary intake of calcium may be a risk factor for periodontal disease. The relationship between calcium and periodontal disease is likely due to the role of calcium in building density in alveolar bone that supports teeth. The objective of this study was to examine the role of calcium supplementation to prevent progression of periodontal disease in a population with medication induced salivary hypofunction.

Methods: Seven hundred and thirty eight volunteers (57% males) were enrolled in this prospective study. The study population had a mean age of 63.7 years, with a mean number of 23.1 teeth and a mean unstimulated salivary flow rate of 0.06ml/min. A calcium supplement questionnaire was administered. Periodontal disease was measured by pocket depth (PD) and gingival recession at six sites per tooth. The periodontal measures were recorded at baseline and nine months. The study population was divided into two groups, one supplementing with calcium and the other not supplementing with calcium, based on self reported calcium supplementation data obtained from the questionnaire, and were compared using a Mann Whitney test.

Results: In the calcium supplementing group, the mean (SD) percentage of sites with PD≥5 mm increased from 1.47 (5.61) % at baseline to 2.18 (7.26) % at nine months. For the group not supplementing with calcium the respective values were 1.83 (4.52) % and 3.36 (6.69) %. On comparing the two groups, the group not supplementing with calcium showed a statistically significant increase in the percentage of sites with PD≥5mm (p=0.005, MW). The mean (SD) increase for the group not supplementing with calcium was 1.51 (6.18) % and for calcium supplementing group was 0.70 (5.15) %. In addition, the groups were evaluated for gingival recession. In the calcium supplementing group, the mean (SD) number of sites with gingival recession increased from 0.28 (0.38) at baseline to 0.60 (0.46) at nine months. For the group not supplementing with calcium the respective values were 0.27 (0.33) and 0.67 (0.52). On comparing the two groups, the group not supplementing with calcium showed a statistically significant increase in the number of sites with gingival recession (p=0.048, MW). The mean (SD) increase for the group not supplementing with calcium was 0.39 (0.37) and for the calcium supplementing group was 0.32 (0.36).
Conclusions: The results from our study suggest that supplementation with the Recommended Adequate Intake of calcium (developed by the Institute of Medicine of the National Academy of Sciences) results in a significant decrease in the percentage of sites with pocket depths $\geq 5$mm and in the number of sites with gingival recession. Our study shows that calcium supplementation may prevent the progression of periodontal disease in a population with medication induced salivary hypofunction.
Title:
Vitamin A Value of High β-carotene Yellow Maize

Authors:
Tawanda Muzhingi, Andrew Siwela, Henry Gadaga, Micheal Grusak, Guangwen Tang

Presented by:
Tawanda Muzhingi

Departments:
Carotenoids and Health Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University; Department of Applied Biology and Biochemistry, National University of Science and Technology, Bulawayo, Zimbabwe; Nutrition Department, National University of Lesotho, Maseru, Lesotho; Agricultural Research Services, Baylor College of Medicine

Abstract:
Background: Vitamin A deficiency is a public health problem in many countries that have maize as a staple food. Yellow maize naturally contains β-carotene. The bioconversion of yellow maize β-carotene to vitamin A in humans is unknown.

Objective: To determine the vitamin A value of yellow maize β-carotene in humans.

Design: Yellow maize was grown in a 23 atom% 2H2O cultural solution. Yellow maize β-carotene showed the greatest enrichment as [2H9] β-carotene. Eight healthy Zimbabwean men with a mean (±SD) serum retinol concentration of 59.2 (17.1) µg/dL and a body mass index (in kg/m2) of 22.4 (3.1) volunteered and adhered to low carotenoid and vitamin A diets. On day 1, after a fasting blood sample was drawn, subjects consumed 300g of yellow maize porridge containing 1mg β-carotene and 20g of fat together with a 170mg oil capsule. On day 8, after a fasting blood sample was drawn, subjects consumed a capsule containing 1mg [13C10] retinyl acetate and 170mg oil, together with 300g of white maize porridge with 20g of fat. Thirty-six blood samples were collected from each subject over a span of 36 days. Concentrations and enrichments of retinol and β-carotene in dose and serum were determined by using HPLC, GC/MS and LC/MS.

Results: The area under the curve (AUC) for deuterium labeled retinol from yellow maize was 72.9 nmoles x day and 161.1 nmoles x day from 1mg 13C10 retinol. The conversion factor of maize β-carotene to retinol by weight was calculated as 2.6 ± 1.0 to 1 , and was significantly correlated with age and serum concentration of retinol (p<0.05).

Conclusion: In eight healthy Zimbabwean men, 1mg of yellow maize β-carotene consumed with 20g fat produced 0.39mg of retinol in the circulation.
Title: Development of a Bacillus subtilis-Based Rotavirus Vaccine

Authors: Sangun Lee, Boris Belitsky, Kathy O'Day Kerstein, James Brinker, Abraham L. Sonenshein, David Brown, Saul Tzipori, John Herrmann

Presented by: Sangun Lee

Departments: Department of Biomedical Sciences, Cummings School of Veterinary Medicine; Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract: Bacillus subtilis spores have been considered as a useful vaccine vehicle due to their safety and stability. In this study we developed a B. subtilis construct expressing rotaviral VP6 and evaluated its vaccine efficacy. B. subtilis was constructed to express either bovine or murine rotavirus VP6 in the cytoplasm of vegetative cells and to display rotavirus VP6 on the spore surface. The B. subtilis spores displaying rotavirus VP6 (VP6 spores) were harvested and used to immunize Balb/C mice. The mice were immunized by intranasal administration 3 times biweekly with 3x10⁹ spores per dose. To enhance mucosal immunity, whole cholera toxin (CT) or E. coli LT (R192G) were included as adjuvants. To evaluate vaccine efficacy, the immunized mice were challenged orally with EDIM virus and monitored daily for 7 days for virus shedding in feces. Mice immunized with VP6 spores (both murine- and bovine-derived VP6) had significantly increased serum anti-VP6 ELISA titers, while the mice immunized with control spores had no serum antibody titers against VP6. Mice in groups that were immunized with VP6 spores plus cholera toxin or LT (R192G) showed a significant reduction in virus shedding, while the mice groups immunized with VP6 spores alone showed no difference in virus shedding compared with mice immunized with control spores. These results demonstrate that Bacillus subtilis-based rotavirus vaccines are effective in generating protective immunity against rotavirus challenge in the animal model used.
Title:
Enterocyte Calpain and *Escherichia coli* O157:H7 Effectors Control Host-Pathogen Interactions: Regulation of Microvillar Effacement, Tight Junction Stability and Epithelial Integrity

Authors:
Kathleen Riley, John Leong, Ira Herman

Presented by:
Ira Herman

Departments:
Department of Physiology, Tufts University School of Medicine; Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School

Abstract:
Infection of enterocytes by enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 results in microvillar effacement, tight junction (TJ) disruption and actin pedestal formation beneath the bound bacterium. Effacement and pedestal formation alter the apical cytoskeleton, whereas TJ disruption affects the basolateral membrane domain. These two membrane-cytoskeletal domains are physically linked through the terminal web, a complex array of filamentous actin and associating proteins. Interestingly, the cytoskeletal-membrane linker protein, ezrin, is both a calpain substrate and a regulator to enterocyte cytoskeletal integrity presumably by linking F-actin to the plasma membrane. We show here that ezrin is cleaved in a calpain-dependent manner during EHEC infection, and that calpain activity is required for enterocyte effacement. We also observe a calpain-dependent repositioning of cingulin, a TJ-associating protein that links the apically disposed cytoskeleton and TJ together. Notably, Tir and EspFu, translocated EHEC effectors required for actin pedestal formation, are also required for ezrin cleavage, microvillar destruction and cingulin relocation. These results suggest that effacement and TJ disruption are mechanistically related and linked to pedestal formation, and that EHEC perpetuates a comprehensive assault on both the apical and basolateral cytoskeletal domains through a common calpain-dependent signaling pathway.
Title:
Nutritional Status in HIV-Infected Drug Users in Hanoi, Vietnam and Chennai, South India

Authors:
Kimberly Dong, Ramakrishnan Ramachandran, Hien Duc Nguyen, Thota Venkata Rao, Lien Trinh,
Tarun Bhatnagar, Dang Van Duong, Kartik Krishnan, M. Suresh Kumar, Mohan Gupte, Vijaya Kumari,
Heidi Sheehan, Hanh La, Alice Tang, Christine Wanke

Presented by:
Kimberly Dong

Departments:
Department of Public Health and Community Medicine, Tufts University School of Medicine; National Institute of Epidemiology, Indian Council of Medical Research, Chennai, India; National Institute of Infectious and Tropical Diseases, Hanoi, Vietnam; Hopers Foundation, Chennai, India

Abstract:
Background: Nutritional complications are frequent in HIV infection and in drug users (DU). Associated morbidity suggests need for intervention.

Methods: We examined nutritional status of male DU in Hanoi, Vietnam (n=197) and Chennai, South India (n=300) who were HIV-positive/HAAART-naïve or HIV-negative. Asterisks in table highlight statistically significant (p< 0.05) differences by HIV-status within site using t-tests.

Results: Participants in Hanoi had a mean age of 31, while mean age was 37 in Chennai. Thirty-one percent of the DU in Hanoi had been incarcerated, as had 68% of DU in Chennai. Seventy-one percent of the DU in Hanoi were married and 99% acknowledged heterosexual sex; in Chennai 41% were married and 91% were heterosexual. DU in Hanoi was mostly heroin and in Chennai, heroin plus prescription drugs were common. Malnutrition is common in DU regardless of HIV-status. BMI, percent body fat, total cholesterol were low. HDL and protein intake were high in Hanoi, even with advanced HIV, but low in Chennai. Triglycerides were elevated in Hanoi, but not in Chennai. Food insecurity was rare in Hanoi and common in Chennai.
### Nutritional status of drug users by HIV and Site

<table>
<thead>
<tr>
<th></th>
<th>Hanoi HIV+ n=99</th>
<th>Hanoi HIV– n=98</th>
<th>Chennai HIV+ n=107</th>
<th>Chennai HIV– n=193</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Laboratory Results</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 (mm$^3$)</td>
<td>97 ± 68</td>
<td>NA</td>
<td>344 ± 188</td>
<td>NA</td>
</tr>
<tr>
<td>Total Chol (mg/dl)</td>
<td>149 ± 40*</td>
<td>179 ± 39*</td>
<td>131 ± 34*</td>
<td>152 ± 39*</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>175 ± 128</td>
<td>161 ± 114</td>
<td>95 ± 45*</td>
<td>90 ± 47*</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>45.5 ± 20.5*</td>
<td>62.5 ± 15.2*</td>
<td>35 ± 11*</td>
<td>41 ± 11*</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>71.0 ± 34.8*</td>
<td>86.7 ± 33.6</td>
<td>81 ± 22*</td>
<td>95 ± 28*</td>
</tr>
<tr>
<td>Insulin Res. QUICKI</td>
<td>0.423 ± 0.12</td>
<td>0.437 ± 0.16</td>
<td>0.365 ± 0.04</td>
<td>0.353 ± 0.05</td>
</tr>
<tr>
<td>QUICKI &lt;0.357</td>
<td>26%</td>
<td>28%</td>
<td>37%*</td>
<td>54%*</td>
</tr>
<tr>
<td>Hepatitis B exposed†</td>
<td>100%</td>
<td>100%</td>
<td>92%</td>
<td>83%</td>
</tr>
<tr>
<td>Hepatitis C exposed</td>
<td>92%*</td>
<td>52%*</td>
<td>92%*</td>
<td>64%*</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>19.1 ± 2.1*</td>
<td>20.5 ± 2.9*</td>
<td>18.6 ± 1.9*</td>
<td>19.1 ± 3.0*</td>
</tr>
<tr>
<td>BMI &lt;18.5</td>
<td>40%*</td>
<td>24%*</td>
<td>52%</td>
<td>48%</td>
</tr>
<tr>
<td>Body fat %</td>
<td>11.2 ± 6.3</td>
<td>11.1 ± 10.2</td>
<td>6.5 ± 4.0*</td>
<td>7.9 ± 5.4*</td>
</tr>
<tr>
<td>Body fat kg</td>
<td>6.2 ± 4.0</td>
<td>6.9 ± 7.0</td>
<td>3.3 ± 2.4*</td>
<td>4.5 ± 3.8*</td>
</tr>
<tr>
<td><strong>Dietary Intake‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>2222*</td>
<td>1957*</td>
<td>2305</td>
<td>2592</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>299*</td>
<td>238*</td>
<td>380</td>
<td>398</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>102</td>
<td>92</td>
<td>59.3</td>
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<tr>
<td>Fat (g)</td>
<td>62</td>
<td>56</td>
<td>58*</td>
<td>71*</td>
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<tr>
<td>Fiber (g)</td>
<td>9.6*</td>
<td>7.9*</td>
<td>4.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*: Difference significant within site
†: Definition of Hepatitis B exposed defined as positive to at least one: HBsAg; HBcAb; HBsAb
‡: Vietnam data based on 72 recalls for HIV+ and 90 recalls for HIV–

**Conclusions:** Vast differences in lipid abnormalities and food insecurity between countries emphasize that interventions to improve nutritional and metabolic status need to be site specific.
Title: Nutritional Supplementation in HIV-Infected Individuals in South India

Authors: Soumya Swaminathan, Padmapriyadarsini Chandrasekaran, Sukumar Balushetty, Sheikh Iliyas, Karthipriya Jeyachandran, Gomathy Paraman, Beena Thomas, Minnie Mathew, Lee Yoojin, Christine Wanke

Presented by: Padmapriyadarsini Chandrasekaran

Departments: Department of Public Health and Community Medicine, Tufts University School of Medicine; Department of Clinical Research, Tuberculosis Research Centre, Chennai, India; United Nations’ World Food Programme, Italy

Abstract:

Background: Malnutrition in HIV-infected individuals results in suboptimal response to antiretroviral therapy, disease progression and a higher mortality rate.

Methods: We conducted an interventional study, to evaluate the effects of a high-protein, high-calorie oral supplement, on the anthropometry, body composition and immune status of HIV-infected adults, between 2005–2007, at the Tuberculosis Research Centre, India. After the initial nutritional assessment, patients were given the supplement and assessed clinically every month by 24-hour dietary recall. Anthropometry, body composition, blood chemistry, and immunology were measured at the 6th month. The outcomes assessed were changes in the nutritional status in terms of anthropometry, blood chemistry, immune status, and body composition.

Results: Among the 371 who completed 6 months of follow up, 288 received the supplement and 83 standard of care. The mean age was 31.5 ± 7.3 years and 42% were males. After 6 months of supplementation, a significant increase in protein consumption, body weight, body mass index, mid arm circumference, fat free mass, and body cell mass was seen in the supplemented group with moderate to severe immunosuppression, but not in controls. There was a significant decline (p<0.05) in the serum albumin and CD4 cell count in the control group, while they remained unchanged in the supplemented group.

Conclusions: High calorie, high protein oral nutritional supplement in HIV-infected patients results in a significant improvement in body mass index, body composition and immune function. Such interventions are likely to have a major impact in resource limited, HIV high burden countries where access to antiretroviral therapy is low.
Title:
Screen for Chromosomal Virulence Factors in *Yersinia pseudotuberculosis*

Authors:
Gregory Crimmins, Ralph R. Isberg, Joan Mecsas

Presented by:
Gregory Crimmins

Department:
Department of Molecular Biology and Microbiology, Tufts University School of Medicine

Abstract:
The genus *Yersinia* comprises 11 Gram negative species, 3 of which are well known for their ability to cause disease in humans: *Yersinia pestis*, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. These 3 pathogenic *Yersinia* species all share a common essential virulence plasmid. This virulence plasmid encodes a type III secretion system (TTSS) and several effector proteins that are translocated through the TTSS into host cells, where they manipulate and inhibit various host cell functions. The *Yersinia* TTSS and its secreted effectors have been the subject of intense study. However, mutant *Yersinia pseudotuberculosis* (Yptb) completely lacking the virulence plasmid are still capable of growth and persistence in internal organs, albeit at a much reduced level. This surprising result strongly suggests that there are a number of virulence factors encoded by Yptb that have yet to be discovered. Therefore, we have set up a screen to identify Yptb genes that are required for growth and persistence in the absence of the virulence plasmid.
Title:
Getting Your Research Done with UIT Services

Author:
Lionel Zupan

Presented by:
Lionel Zupan

Department:
University Information Technology (UIT) Academic Technology, Tufts University

Abstract:
Dr. Lionel Zupan will give an overview of the research technology services provided by University Information Technology (UIT) to the Tufts research community. He will also discuss how Tufts faculties are using research technology in their work. UIT services currently include: research high performance computing, research network storage, software network licensing, tools for visualization, statistical consulting, technology consultation and planning, monitoring, design, implementation and application of emerging technologies for research applications.
Title:
A Unique Research Resource: The Tufts Clinical and Translational Science Institute

Authors:
Ira Wilson, Andrew Plaut, John Griffith

Presented by:
Anne Kane

Department:
The Tufts Clinical and Translational Science Institute

Abstract:
The mission of the Tufts Clinical and Translational Science Institute (CTSI) is to identify, stimulate, and expedite innovative clinical and translational research with the goal to improve the public's health.

The Tufts CTSI provides education, training, and research support to investigative teams from all walks of research life – the bench laboratory, clinical research, healthcare delivery, and public policy – leveraging Tufts University's traditions of multidisciplinary collaboration, novel research methods, and academic innovation. We salute the Global Health Research efforts of the Tufts community and extend our resources to all its investigators.

The aim of the Tufts CTSI Portal is to support researchers in conducting innovative clinical and translational research. The Portal encompasses eleven components, including the Design and Database Resource Center, Novel Clinical and Translational Research Methods Development, Translational Technologies and Resources, Therapeutics Development and Implementation, Predictive Medicine, Evidence-based Medicine, Genetics and Genomics, and a Pilot Project Program. Through the Portal, investigators can:

- Find research resources
- Connect with other researchers, industry, and community partners
- Participate in educational programs and training opportunities
- Access consultation on how to design, implement, and execute a study
- Identify funding opportunities for clinical and translational research locally and nationally

The Portal’s focus is on providing infrastructure and processes that support researchers every step of the way from developing an idea to obtaining funding to implementing a project to disseminating findings and influencing health care policy. Contact us at www.portal.tuftsctsi.org.
Title:
Tufts Animal Pathology and Histology Core Laboratory Services

Authors:
Lauren Richey, Brian Lagace, Derek Papalegis

Presented by:
Lauren Richey

Department:
Division of Laboratory Animal Medicine, Tufts University

Abstract:
The Tufts Research Animal Health and Pathology Support (RAHPS) laboratory provides pathology and histology support for investigators working with research animals. A board certified veterinary pathologist trained in the comparative pathology, physiology, health, strain variation, and phenotypic analysis of animals is able to assist researchers at every step of their projects, from model selection, to sample collection, lesion documentation, and interpretation of findings. Services include gross necropsy examination, photodocumentation of lesions, histology by a certified histotechnician experienced in research animal tissues, review of slides by the veterinary pathologist, consultation for pathology study design, review of pathology data and reports, training in pathology techniques, and assistance with clinical pathology needs. The RAHPS laboratory supports the research of Tufts University and Tufts Medical Center investigators by working with each person to meet individual research pathology and histology needs.