EGH Research Faculty Go Global

The Research Faculty at EGH are busier than ever with projects ongoing in Haiti, Kenya, Mongolia, Thailand, and more... continued on p.2

PhD Program Set to Begin!

Fall 2011 marks the start of the Department's new PhD program in Environmental and Global Health. The new cohort of students will work closely alongside EGH faculty researchers, receiving both classroom instruction and crucial hands-on training. ...continued on p.11

This year marked the fourth annual Certificate Program in Emerging Infectious Disease Research (CEIDR) for the Department of Environmental and Global Health. Each year, the Certificate Program provides specialized graduate level education to nominated international, public health professionals in the area of emerging infectious disease research. Participants spend two and a half weeks of intensive training at the University of Florida studying topics such as epidemiology, biostatistics, zoonotic diseases, entomology, microbiology, water quality, and food safety. Students of 2011 CEIDR Program received sponsorship from the US Department of Defense’s Armed Forces Health Surveillance Center and the US Department of State.

The thirty-five international students who took part in this year’s program came from fifteen different countries: Cambodia, Cameroon, Egypt, Georgia, Kenya, Malaysia, Mongolia, Nepal, Nigeria, Peru, Philippines, Poland, Romania, Thailand, and Uganda. The Certificate Program provides students with advanced training, which is often unavailable in their home countries. In addition to classroom-based learning, students enjoy several educational trips to witness modern food production and learn entomological techniques.

After another successful end to classes in Gainesville, the students returned to their home countries to continue their studies online. The Department looks forward to ongoing research and future collaborations with a tremendous group of public health and veterinary professionals!
Research on the Epidemiology and Control of Locally-acquired Dengue Fever in 2009 and 2010 in Key West, Florida

While dengue infection in residents returning from endemic locations abroad is not uncommon, locally-acquired dengue, such as that reported for Key West, Florida in 2009 and 2010, certainly is. Not since 1945 have locally-acquired dengue infections been reported in the continental United States, other than along the Texas-Mexico border. The last dengue outbreak in Florida occurred in 1934.

Between early July and mid-October 2009, twenty-seven cases of locally-acquired dengue were reported by residents or visitors of Key West. After a six-month lull, dengue cases resurfaced again. By the end of 2010, more than 60 cases were reported in Key West and one additional case was reported in Broward County.

Continuing local and intermittent transmission, in spite of various attempts at vector control during 2009 and 2010, indicates that the basic reproductive number of $R_0$ (measure of transmission) in local dengue transmission continues. We were able to estimate transmission thresholds in terms of *Aedes aegypti* adults per household by using historical average monthly temperatures and assuming a seroprevalence of antibody against dengue to be 10%. Extensive adult trapping data in the areas of transmission by the Florida Keys Mosquito Control District (FKMCD) indicate levels several times that of estimated thresholds in 2009 and 2010. Pupal and demographic surveys have been conducted by FKMCD, but the number of *Ae. aegypti* pupae observed is inconsistent with adult densities.

We have concluded that cryptic breeding in unmonitored and/or undetected, abandoned water storage cisterns and septic tanks may account for the excess production. We have recommended an exhaustive survey for these cryptic sites in order to control them. Additionally, FKMCD surveyors now have access to historical fire-insurance maps of Key West, which show locations of cisterns.

By utilizing commercial airline data between 2007 and 2010, we were able to estimate the magnitude of potential introductions of dengue viruses into Key West. The question remains as to why Key West experienced locally-acquired dengue in 2009 and 2010, but not in previous years and decades. We obtained the number of airline passengers with originating flights in dengue-endemic countries in this hemisphere and a final destination of Key West, Florida between 2007 and 2010. We then subsequently converted the data using year- and country-specific incidence rates for dengue.

Results indicate that Caribbean countries are responsible for the majority of potential dengue introductions during this time. Additionally, the data confirmed that the magnitude of introductions was indeed highest between 2009 and 2010. This is reflective of both rising passenger numbers and increased dengue prevalence in the Caribbean basin, which was due to temperature anomalies associated with El Nino/Southern Oscillation conditions.

This work will be continued this summer with an examination into the number of passengers and crew with a port of call in Key West, Florida between 2007 and 2010.

Dana A. Focks, Kelli L. Barr, and Ali Messenger
Dr. John Lednicky to Conduct Research on Airborne Pathogens

At the University of Florida, Dr. John Lednicky is setting up an Aerobiology Laboratory for inhalation exposure studies of BSL2- and BSL3-pathogens. A special system is used and allows for precise delivery of pathogens as droplet-nuclei aerosols (<5 micron diameter).

Dr. Lednicky will use aerobiology to investigate the relationship between inhaled dose pathogens and clinical outcomes/pathology posed by airborne pathogens. He also investigates the genetic basis of virulence among different virus isolates.

Dr. Lednicky has studied numerous pathogens and is experienced in diagnostic, applied, and research microbiology. Additionally, Dr. Lednicky teaches advanced courses in environmental health at the University of Florida.

Access to one of Dr. Lednicky’s recently published articles on the nose-only inhalation exposure system can be found here: http://www.virologyj.com/content/7/1/231/abstract

Studies of respiratory pathogens delivered through the more natural inhalation exposure route instead of by intranasal instillation or intraperitoneal injection offer various advantages, including:
- Clinical signs and pathology are more like those observed in natural infections
- Disease is often exacerbated, and occurs earlier
- Contamination hazards are drastically reduced
Studies to Assess Toxicity of Nanomaterials in Plants

Story by Tara Sabo-Attwood

Nanomaterials are a potential novel class of pollutants that need to be assessed for their environmental fate and adverse effects to ecosystem and human health. Since the acute and long term fate and toxicity of nanoparticles to flora are unknown, determining how they interact with plants and subsequent biological effects is critical as this can have a significant impact on fate, transport and trophic transfer in ecosystems.

Sabo-Attwood et al., have recently published a paper in Nanotoxicology where they investigated the ability of gold nanoparticles (AuNPs) to enter plants through size-dependent mechanisms, translocate to cells and tissues and cause biotoxicity. Using tobacco plants (Nicotiana xanthi) as a model system they revealed that only 3.5 nm AuNPs entered plants through the roots, formed clusters in root cell cytoplasm (figure 1), distributed into the vasculature (figure 2) and caused leaf necrosis (figure 3), while 18 nm sized particles did not penetrate the roots.

This is the first report to track the biodistribution of AuNPs throughout a plant and observe clustering of these particles around root cell cytoplasmic bodies. These results collectively highlight the need for further studies on the fate and effects of nanoparticles on plant species and further suggest a route of AuNP entry into terrestrial food webs.

Figure 1. High Resolution TEM and elemental analysis showing clusters of 3.5 nm AuNPs within an individual root cell. Scale bar = 0.5 µm

Figure 2. Synchrotron based X-ray fluorescence micrograph of the plant vasculature of the leaves after exposure to 3.5 nm AuNPs showing the presence of gold where the highest concentrations are represented by red-yellow spots.

Figure 3. Tobacco plants exposed to 3.5 nm AuNPs for 14 days show necrosis on the leaves (white areas)
Dr. Richard Rheingans

Research Interests

- Effectiveness, sustainability and scalability of school-based water and sanitation intervention
- Determinants of disparities in water quality and sanitation in peri-urban areas
- Impact and cost-effectiveness of diarrheal control strategies in low-income countries
- Impact of water and sanitation on psycho-social stress

Dr. Rheingans’ research team is currently working on two new projects to characterize disparities in key global health areas and identify effective approaches to reaching those most in need. The research team includes John Anderson, Yilun Sun, Peng Jia, and Matt Kukla. In one of their current projects, which is supported by PATH, Rheingans’ team seeks to estimate the distributional effects of new vaccination programs against rotavirus diarrhea in 23 low-income countries. These disparities can be based on socio-economic and geographic differences.

Recently the Bill and Melinda Gates Foundation and the UK Department for International Development (DFID) announced large multi-year investments to expand access to rotavirus vaccines for the poorest countries. Dr. Rheingans’ work demonstrates that additional efforts are needed to ensure that such programs reach the poorest of the poor, while maximizing benefits and cost effectiveness. Additionally, Rheingans and his team are developing innovative approaches to visualize and show these distributional effects.

In a second project, Dr. Rheingans’ research team is working on a grant from the DFID-funded SHARE Research Consortium to assess disparities in global access, exposure and health risks related to poor sanitation. While current development targets like the Millennium Development Goals aim to improve sanitation coverage, they do not emphasize reaching the poorest populations.

Rheingans’ research uses statistical and spatial modeling to show magnitude of disparities, underlying causes, and the health and economic benefits of targeting sanitation improvements to the poor. The results will feed into SHARE’s broader efforts to redefine how sanitation progress is monitored.

Dr. Thomas B. Waltzek

comes to the Department of Environmental and Global Health after completing his Ph.D. studies on the evolution and ecology of viral diseases of poikilothermic vertebrates. Waltzek received his Masters and Veterinary Medical degrees from the University of California at Davis where he studied the functional anatomy and ecology of cichlid fish and fish health, respectively.

Now a Postdoctoral Research Associate at the Global Pathogens Lab (GPL), Waltzek plays a key role in research activities including the surveillance and characterization of emerging aquatic animal pathogens. In collaboration with members of the UF College of Veterinary Medicine, Waltzek is leading the GPL team on a surveillance project to detect respiratory viral pathogens in captive and wild populations of marine mammals. The team has recently isolated and begun the characterization of a novel adenovirus recovered from a stranded harbor porpoise.

Waltzek has received many accolades for both presentations and papers on a variety of subjects from fish physiology and ecology to infectious diseases of fish and marine mammals. Waltzek was recently voted the newest member of the Aquatic Animal Veterinary Medicine Committee for recognition of his expertise in public health/epidemiology.
Seroepidemiological Study of Bovine Zoonoses in Persons Occupationally Exposed to Cattle

Dr. Afsar Ali

Dr. Ali conducts research on the epidemiology, ecology, evolution and disease transmission of cholera and its causative agent, *Vibrio cholera*. Ali’s research is particularly focused in developing countries where cholera causes massive scourge in humans.

During the recent cholera epidemics in Haiti, Ali successfully determined that the circulating *V. cholerae* strains were highly clonal. Ali discovered that the clonal nature of these strains would cause them to diversity as they passed through Haitian populations and presumably water reservoirs. This is despite the fact that no *V. cholerae* strains were isolated from aquatic reservoirs in Haiti. Ali and his team of researchers are preparing to monitor various environmental sites in Haiti. They seek to isolate *V. cholerae* and examine whether or not the environmental and clinical strains are genetically similar. Ali’s findings were published in the April 2011 issue of the *Journal of Emerging Infectious Diseases*.

Additionally, Ali’s team collected sixteen water samples from various aquatic reservoirs in Haiti, including ponds, rivers, and estuaries. Upon laboratory examination, 81% of the aquatic reservoirs harbored either culturable or genetic determinants of non-O1 *V. cholerae* strains. These findings suggest that the Haitian aquatic reservoirs were conducive to promoting endemic cholera even before the sudden cholera epidemic in 2010. On another front, Ali reported that *Vibrio vulnificus* (another deadly human Vibrio pathogen) is highly colonized in tilapia fish farmed in coastal area of Bangladesh. All isolated strains are 100% genetically linked to clinical strains of global origin rather than to environmental isolates. This work has also been reported in Applied and Environmental Microbiology (2010).
Researchers Find Evidence of Cryptic Avian Influenza Infections among Rural Villagers in Thailand and Cambodia

Global Pathogens Laboratory team researchers, along with collaborators from the U.S. Naval Medical Research Unit #2 (NAMRU2), US Armed Forces Research Institute of Medical Sciences (AFRIMS), and ministries of health in Thailand and Cambodia have been collaborating for several years in a project to prospectively study 1600 rural villagers in Thailand and Cambodia with intense exposure to poultry for evidence of avian influenza (AI) virus infections. Serological analyses of participants' sera collected during the enrollment period suggest enrollees had elevated antibodies by intense exposure to poultry for evidence of avian influenza (AI) virus infections. Serological analyses of the sera are ongoing. Prospective data from this project will help us better understand the serology of avian influenza infections in a rural cohort in SE Asia.

(Funded by grants AFSHC-GEIS I0179_10_UN and NIH/NIAID-1 RO1 AI068803-01. Gregory C. Gray, Principal Investigator.)

Dr. Kalina Atanasova is a post-doctoral researcher under the guidance of Dr. Greg Gray at the Global Pathogens Laboratory of the Emerging Pathogens Institute. She received her veterinary master's degree at the University of Forestry in Sofia, Bulgaria and her PhD from the Laboratory at Ghent University. During her studies, Dr. Atanasova's research focused mostly on porcine respiratory diseases and, in particular, the inflammatory effects of simultaneous exposure of porcine lungs to porcine respiratory coronavirus and bacterial cell-wall toxins. During her PhD she also performed studies on tissue distribution of swine influenza virus in pigs. This research sparked her interest in the unfamiliar field of influenza infections in the nervous system and the routes of influenza transmission to the central nervous system among different natural hosts. Currently, Dr. Atanasova is trying to better understand how influenza A virus infects and spreads in nervous tissue. She also continues her research on other porcine zoonoses. Atanasova's recent projects include a sero-survey of human sera for antibodies against porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Dr. Atanasova is also establishing in vitro models for neuronal infections of influenza viruses in pig and chicken primary neurons.

An emerging infectious disease first seen in persons with intense dog exposures could indicate a canine pathogen has spread across species. Identifying specific disease risks in this occupational group would bring more awareness of zoonotic diseases to persons who are occupationally exposed to dogs.

Using an informed consent process, healthy adults whose work or hobbies involved exposure to dogs completed an enrollment questionnaire and permitted a serum sample collection. Targeted populations included breeders, veterinarians, and shelter, kennel and racetrack workers. Persons not exposed to dogs in the last 5 years were enrolled as a control group. In total, 306 dog-exposed workers and 101 non-exposed controls were enrolled and provided a sera sample. Laboratory analyses of the sera are ongoing.
The primary focus of Dr. Bernard Okech’s ongoing research is related to mosquito vectors and the diseases they transmit. Dr. Okech studies the biological processes occurring in the mosquito midgut after food ingestion and how those processes influence mosquito survival and disease transmission. Okech is currently looking at malaria transmission and breeding habitats for dengue mosquito vectors in Haiti.

Dr. Okech has partnered with Ministry of Health officials in Haiti to characterize anti-malarial drug resistant genotypes throughout the country. Okech’s malaria transmission research in Haiti focuses on anti-malaria drug resistance, malaria mosquito vector breeding habitats, and biting activity. To date, drug resistant malaria infections in Haiti have not been well documented due to poor surveillance efforts.

Dr. Okech is also evaluating environmental factors associated with dengue vector mosquito breeding in urban and peri-urban areas in Haiti. In a community near City Soleil in Port Au Prince and also in the town of Gressier, Dr. Okech maps out breeding sites of Aedes albopictus, Ae. mediovitattus and Ae. aegypti. At his laboratory at the University of Florida’s Emerging Pathogens Institute, Dr. Okech studies mechanisms of nutrient transport in the Aedes aegypti midguts with the goal of elucidating unique pathways of nutrient absorption that can be exploited for mosquitocide development.

Dr. Amanda Rice, postdoctoral associate, is focusing on the free living pathogenic amoebae Naegleria fowleri, Acanthamoeba spp. and Balamuthia mandrillaris that can be found in warm fresh water environments. These amoebae are opportunistic pathogens that may cause lethal central nervous system infections. The most well known of the three in Florida is Naegleria fowleri, the causative agent of primary amoebic meningoencephalitis (PAM). In collaboration with the Florida Department of Health and the Centers for Disease Control and Prevention, Dr. Rice is currently researching the environmental conditions in which free living pathogenic amoeba thrive and the specific host immune responses that control the infections of these amoebae.

To date, environmental data to determine the key factors required for the amoebae, specifically N. fowleri, to reach levels ideal for human infection have not been determined. Utilizing the collected data, this study will produce invaluable information for the modeling to determine conditions that lead to high numbers of amoebae in recreational fresh waterways. This research and its new collaboration will provide critical support and further direction for public health campaigns in controlling or eliminating amoebae from water sources throughout the state of Florida. There have been 118 cases of PAM since 1962 in the United States with only a single patient surviving the infection. Currently the type of immune response that is responsible for determining the outcome of an individual infected with N. fowleri is not well understood. By studying individuals with long term exposure to water containing the amoebae, Rice will be able to better understand the immune responses that protect individuals from a lethal infection. Concurrently, the disease process and the development of alternative treatment regimens leading to a higher survival rate will be evaluated using the information gained from the study of individuals with long term exposures.
The Global Pathogens Laboratory hosted three international scholars for four weeks of training in influenza laboratory techniques. The three trainees, Urushadze, Rapuntean, and Myagmarsuh, learned influenza culturing techniques, molecular methods used for characterization of influenza specimens, and serological methods for identifying antibodies against influenza in human sera. Support for this training was provided by a grant from NIAID CEIRS network. Upon completion of the training, the visiting scholars were presented with a certificate and letter highlighting the training they received at the University of Florida.

**Epidemiological Study of Persons with Intense Exposure to Ruminants for Rift Valley Fever Virus Infection**

Rift Valley Fever (RVF) is a zoonotic arbovirus with the majority of human infections resulting from direct or indirect contact with infected animal blood or organs. The virus may also be transmitted to humans through the handling of animal tissue during slaughter, butcher, or veterinary procedure. Transmission to humans occurs through inoculation with broken skin or inhalation of aerosols produced during slaughter. Certain occupational groups such as herders, farmers, slaughterhouse workers, and veterinarians are thus at a higher risk of infection. Human infections can also be vector-borne, via bites of infected mosquitoes, ticks, or flies. In irrigated areas where mosquito populations are prevalent this mode of transmission is more frequent. Infection can cause severe disease in both animals and humans, and ultimately lead to high rates of abortion (animals), disease, and death. The disease also results in significant economic loss through the death and abortion of RVF-infected livestock.

Little is known about the risk factors of RVF infection among humans. Other serological studies have been constrained to small geographical areas. For the first time, a risk factor analysis is being conducted in collaboration with investigators at Kenya’s International Livestock Research Institute, Madagascar’s Institut Pasteur and Saudia Arabia’s Ministry of Health. We are performing a controlled cross-sectional pilot study of 1000 adults, living within four distinct geographical sites, with recent, previous RVF infections in animals to look for evidence of previous RVF infection in humans. Collaborators in each of the sites are enrolling adults with extensive exposure to camels, cattle, deer, goats, pigs, or sheep.

Risk factor data from this study will be used to design more comprehensive epidemiological studies, guide the use of diagnostic tools, and identify geographical areas for RVF interventions. Identifying subclinical and unapparent infections in a highly exposed population will, not only allow for a better understanding of cross-species transmission of this zoonotic pathogen, but also guide development and deployment of prevention and control strategies.

Kelli L. Barr, Gregory C. Gray, John A. Friary

Grant: Department of Homeland Security “Epidemiological Study of Persons with Intense Exposure to Ruminants for Rift Valley Fever Virus Infection”
Prevalence of Myobacteria in Florida’s Waterways

Story by Andrew Kane

*Mycobacterium* is a genus of bacteria best known as the causative agents of tuberculosis and leprosy (*Mycobacterium tuberculosis* and *M. leprae*, respectively). The vast majority of mycobacteria, however, are free-living environmental species that can be opportunistic pathogens. A variety of mycobacterial species are associated with wildlife (including fish, bats and elephants, to name a few), and in some cases, mycobacteria can be spread from animals to humans.

Studies at the University of Florida’s Aquatic Pathobiology Laboratories have focused on mycobacterial infections in fish and humans. Researchers are now beginning to look at environmental factors that may foster the presence of these microorganisms in certain environments. Recent field efforts, using novel real-time PCR probes, have demonstrated the ubiquitous distribution of these environmental mycobacteria, including species of mycobacteria known to cause human diseases in surface and drinking waters in the southeastern United States. “Finding these mycobacteria in drinking water is not surprising since many of these bacteria are resistant to chlorination,” said Dr. Andrew Kane, Director of the Aquatic Pathobiology Laboratory. “We are now trying to determine the environmental factors that might be making some of these microorganisms more dense in certain waterways, and what might foster virulence and differential susceptibility in human and animal hosts.” Dr. Kane is working with clinicians from the Southeastern National Tuberculosis Center and other researchers from the University’s Emerging Pathogens Institute, who have expertise in geographic information systems (GIS), epidemiology, molecular biology, water quality and environmental health. Dr. Kane and collaborators will begin unraveling some of the questions pertaining to how people and animals become infected. Ultimately they seek to determine why Floridians and other residents throughout the Southeast may be more susceptible to environmental mycobacteria infections than residents of other areas in the United States.

Mycobacterial disease in fish may or may not include external ulcerative lesions (Fig.1) or the presence of granulomas (Fig.2). Environmental mycobacteria are associated with skin diseases (Fig.3) that can be associated with handling fish or water contact. Pulmonary disease, as seen in the CT scan (Fig.4), is one of the more common clinical manifestations of environmental mycobacterial disease in the southeastern United States. Osteomyelitis, lymphadenitis, corneal infections and other chronic diseases can also be associated with environmental mycobacteria.
New! PhD in Environmental and Global Health

The Department of Environmental and Global Health is proud to host its first cohort of PhD students starting Fall 2011. The new PhD in Public Health, offered by the College of Public Health and Health Professions, will require a minimum of 90 post-baccalaureate credit hours. These credits will include core public health coursework (15 credits); quantitative methods and statistics (12 credits); professional issues (7 credits); concentration area (35 credits); supervised research (3 credits); supervised teaching (3 credits); and dissertation research (15 credits). Initial areas of concentration include environmental and global health and social and behavioral sciences. It is expected that additional concentrations will be added over time and that students will also be able to specialize within each concentration. Currently, the department plans to develop a new concentration with a “One Health” focus. This concentration will prepare graduates to lead teams in conducting studies of complex problems involving humans, animals, and the environment.

Upon successful completion of all program components, culminating in the dissertation defense, program graduates will be awarded the PhD in public health. Examples of places of employment include universities, federal and state government agencies (e.g. Centers for Disease Control, public health departments), health and environmental research firms, and non-profit local, national, and international agencies.

For more information, please visit http://egh.phhp.ufl.edu/academic-programs/phd-program/

All of the faculty and the staff at EGH are looking forward to a great year! The following students have been admitted to the PhD program in Environmental and Global Health.

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Dr. Gray [right] and guest, Dr. Mark Fukuda, during his visit to EGH.

EGH Faculty member, Edsel Redden, conducting research work in Haiti.

Dr. Okech during a recent research trip to Haiti to continue his work on Malaria.

Col. Art Lyons speaking with EGH Faculty and the GPL Team Members at the Emerging Pathogens Institute.

Certificate students examining samples at the Emerging Pathogens Institute.

Swabbing horses for Influenza A virus during Dr. Greg Gray’s recent trip to Mongolia.
Critically-ill child receiving treatment for dengue haemorragic fever in Indonesia.
Photo by Dr. Dana Focks

Drs. Kane and Okech screening for Malaria GPD deficiency and sickle cell anemia at a small clinic in Haiti.

GPL staff members, John Friary and Clint McDaniel, with GPL Intern Diana Cardwell enrolling cattle workers for an ongoing research study.

Modern pig farm in rural China. Photo by Dr. Henry Wan.

Dr. Gregory Gray and Pam McKenzie (St. Jude Children's Hospital) visiting with collaborators during a recent trip to Mongolia.

Dr. Ali distributing Oral Rehydration Saline (ORS) to Saint Mark Hospital in Haiti, where there is a high incidence of Cholera.
Nikki Burke is the latest addition to the EGH team. Nikki is an avid photographer and loves working with students.

David Buckelew works on many of the growing and evolving needs at EGH. David is a graduate of the University of Florida and a true gator fan.

Christa Roberts handles the fiscal needs for EGH and enjoys the fast-paced environment. Christa also put together this newsletter.