2012

ANNUAL REPORT

Joint Institute for Food Safety and Applied Nutrition

The University of Maryland

December 2012

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Mission

JIFSAN advances sound strategies that improve public health, food safety, and applied nutrition using risk analysis principles through cooperative research, education, and outreach programs.

Vision

To be a premier source of scientific information and education programs on food safety and applied nutrition that enables the development of sound public health policy and reduces the incidence of food-related illness.

Joint Institute for Food Safety and Applied Nutrition (JIFSAN)

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From the Director

JIFSAN continued to thrive in 2012. There were several significant accomplishments: the fourth 5-year Cooperative Agreement with the Food and Drug Administration (FDA) was successfully renewed, which allows JIFSAN to enhance its current programs and develop new ones to meet global needs in scientific information on food safety and applied nutrition; JIFSAN has developed a strategic plan to guide its programs and directions and to support the new Food Safety Modernization Act that emphasizes the concept of preventing food safety-related problems before they occur; the International Food Safety Training Laboratory (IFSTL) celebrated its first year, training food safety professionals from over 10 countries.

JIFSAN's efforts in developing sustainable partnerships for training within the global food safety community have also paid off. The Aquatic and Aquaculture Food Safety Center (AAFSC) in Bangladesh, an outgrowth of JIFSAN's capacity building partnerships, has begun offering trainings on its own. Work continues with partners in several countries including India, Mexico and Thailand to establish additional global centers. JIFSAN has also developed tools to measure the effectiveness of our international capacity building training programs.

The Institute continues to strengthen research collaborations in microbial food safety and applied nutrition, and to offer an excellent undergraduate internship program that provides unique opportunities for UM students. Our online distance learning programs have been expanded beyond food safety risk analysis courses to including individual training modules and webinars. We are committed to enhancing JIFSAN programs with new technologies.

I would like to acknowledge JIFSAN faculty and staff, colleagues at FDA, JIFSAN's Advisory Council members, our international partners and other supporters whose efforts enable JIFSAN to continue to build strong programs and make an impact on global food safety and public health.

Jianghong Meng, PhD

Director

STRATEGIC PLAN

JIFSAN has developed a strategic plan that identifies four strategic areas (Appendix B – Strategic Plan) and the following five key objectives. The plan will play an important role in guiding JIFSAN's programs and directions to support the new Food Safety Modernization Act (FSMA) which emphasizes the concept of preventing food safety-related problems before they occur and enhance FDA's efforts to partner with other nations to improve US and worldwide health.

1. To increase the global knowledge of effective, available practices that promote food safety throughout the supply chain.
2. To enhance the development and promote the use of risk analysis models and tools for decision making processes associated with food safety and applied nutrition.
3. To foster collaborative research efforts related to risk analysis, food safety, and applied nutrition.
4. To broaden research educational opportunities for undergraduate and graduate students at the University of Maryland
5. To promote the development of private and public partnerships and international cooperation

CONDUCTING INNOVATIVE RESEARCH IN FOOD SAFETY AND APPLIED NUTRITION

JIFSAN continued to work with scientists at FDA, UM and other organizations to conduct innovative research to address major issues in food safety, applied nutrition, and other areas that impact the public health. JIFSAN's research program assists in the generation of new knowledge supporting the development of a strong scientific base to address ongoing and increasingly complex public health issues. The program includes not only traditional laboratory and field research, but also educational, behavioral and social research. JIFSAN works closely with FDA scientists to identify research needs that bring together researchers at UM, FDA and other institutions to conduct collaborative research. During this reporting period, projects funded through JIFSAN's internal research program and internship program, and research collaborations between JIFSAN and FDA have generated eight publications in peer-reviewed journals (Appendix C). JIFSAN continued building a strong research program with a direct impact on the public health. Research progress of several projects is highlighted below:

<u>Genomics of foodborne bacterial pathogens</u>

- **Comparative Genomics of *Salmonella***

  Nontyphoidal *Salmonella* serovars cause approximately 1.4 million illness cases and several billion dollars in economic losses each year in the United States. *S.* Newport ranks among the top three *Salmonella* serovars associated with foodborne salmonellosis. The pathogen is a polyphyletic organism and the strains within this serovar do not all cluster together in a dendrogram. Horizontal gene transfer occurs frequently in a region around *mutS* gene and the region displays a mosaic genomic structure. We selected this region to determine variations between different lineages of *S*. Newport. The region around *mutS* gene includes *ste* fimbrial operon, Clustered,

Regularly Interspaced, Short Palindromic Repeats (CRISPR) associated protein (*cas*) genes and genes facilitating horizontal gene transfer, including transposase and recombinase. The variation of this region enables us to get a better understanding of evolution process and genetic flux around *mutS* gene. The objective of this study was (1) to explore the evolutionary history of *S*. Newport lineages; and (2) to understand genomic diversity of *Salmonella* Pathogenicity Islands (SPIs) via whole genome sequencing data. The findings suggested that *S*. Newport Lineages II and III diverge early in the serotype evolution and have evolved largely independently. Genes that delineate sublineages within the phylogenetic tree could be used as potential biomarkers for traceback investigations during outbreaks.

- **Non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC)**

  Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in foodborne illnesses causing diarrhea and hemolytic uremic syndrome (HUS) worldwide. Although *E. coli* O157:H7 is the major enterohemorrhagic *E. coli* (EHEC) linked to HUS in the United States, other non-O157 STEC strains have caused several outbreaks and have been isolated in similar frequency. The mechanisms underlying the evolution and emergence of new bacterial pathogens are not well understood. The objective of the study was to elucidate the evolution of virulence and pathogenic mechanisms. The study revealed that an evolutionary relationship might exist between strains with the same flagellar antigen (H) type. This demonstrates that whole genome level sequence analysis is a powerful tool to study STEC and to reveal the evolution relationship between different STEC strains. The findings can lead to the development of diagnostic tools for identifying high risk STEC.

<u>Development and Validation of Isotope Methods for Distinguishing Between Naturally Occurring and Synthetic Phthalates in Food</u>

"Phthalates" (1,2-benzenedicarboxylic acid esters, herein PAE) are used as plasticizers (compounds used to make plastics more flexible), as a solvent for oil-soluble dyes and nitrocellulose lacquers, as an antifoaming agent, a fiber lubricant in the textile industry, a fragrance fixative, and an alcohol denaturant. As such they are used in a variety of consumer products including inks used to print labels on food packaging. However, phthalates are being phased out of many products as they have been identified as reproductive and developmental toxicants, though their toxicity varies depending on the specific phthalate structure. The study was to develop isotope methods that could be implemented by FDA laboratories in detecting synthetic phthalates in food for regulatory purposes.

<u>Metrics for evaluating effectiveness of international food safety trainings</u>

Since 2000, JIFSAN has been involved in developing and conducting training programs for the global food safety community. The goal of these programs is to increase the knowledge and use of best practices that improve the quality and safety of food primarily through "Train-the-Trainer" programs.

The Food Safety Modernization Act (FSMA) aims to ensure the U.S. food supply is safe by shifting focus of federal regulators from responding to contamination to preventing it. This change places a stronger emphasis on monitoring the effectiveness of these programs. JIFSAN researchers have developed a set of process and outcome metrics tools to evaluate individual participants knowledge prior to and immediately upon completion of the training programs. These tools were piloted during recent training courses and preliminary results indicate a high level of knowledge transfer. The immediate goal is to continue to refine these tools in an effort to provide JIFSAN with accurate measurements to assess the effectiveness of its training programs.

TOWARDS SUSTAINABLE INTERNATIONAL FOOD SAFETY TRAININGS THROUGH PARTNERSHIPS

Training programs constitute a significant component of the JIFSAN mission. International training programs are conducted with co-sponsors in the host country. Partnerships with host countries, with industry, and with government agencies have been a key to JIFSAN's success with acquiring resources for expanding the Institute's activities. Programs conducted in 2012 (including December 2011 that was not reported previously) are listed in Appendix E.

The Aquatic and Aquaculture Food Safety Center (AAFSC) in Bangladesh

In the previous report we described the activities surrounding the establishment of this Center in 2009 and subsequent training activities. In the current period an additional training program has been conducted in Bangladesh which was organized and led by local trainers with oversight and input from JIFSAN and FDA consulting instructors. To summarize specific activities, in 2009 Phase I of the program involved a standard JIFSAN training program delivered in Khulna, Bangladesh. In 2010 Phase II was completed with the training of selected outstanding individuals who traveled from Bangladesh to Maryland for intensive training on issues specific to their industry. Later in 2010 Phase III training was conducted in Bangladesh with consulting support from JIFSAN and FDA. Most recently in December, 2011 Phase IV was conducted in Bangladesh with the objective of expanding upon the cadre of individuals with the capability of conducting Train the Trainer programs in Bangladesh so that all segments of the industry will eventually receive critical training. One University (Bangladesh Agricultural University) has incorporated GAqP into its programs and other Universities have expressed similar interest and intentions. The AAFSC is now firmly established as per the plan outlined in the Center Agreement. The success of this Center continues to be a model for the establishment of other training centers as follows in this report.

The Advanced Good Agricultural Practices Training Center in Mexico (GAPTC)

Continued concern for the safety of fresh produce imported from Mexico into the U.S. led JIFSAN to investigate the feasibility of establishing a GAP Training Center in manner similar to the model for the GAqP Center described previously. Preliminary discussions revealed a strong interest from industry associations and government agencies in Mexico. A formal agreement has been approved by the Legal Department at the University of Maryland and has been

forwarded to the Secretaría de Agricultura, Ganadería, Desarollo Rural, Pesca y Alimentación / Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SAGARPA/SENASICA) in Mexico. SENASICA has sent out a solicitation for partnerships with the academic and industry sectors in Mexico. We anticipate that the Agreement will be finalized and that the first training activities will be conducted in early 2013.

Collaborative Training Initiative for Supply Chain Management for Spices and Botanical Ingredients (SCMSB) in India

Outbreaks of illness associated with the consumption of black pepper and perhaps other spice products has heightened the concern for the consumer safety of spices and botanical ingredients. India is a leading supplier of these products to the U.S. and an initiative has been implemented by JIFSAN for providing food safety training to key members of the industry, academia and government in India through the establishment of a Training Center as has been described previously for GAqP and GAP. A Draft of this agreement has been prepared by JIFSAN with input from appropriate persons at the US-FDA. The partners in India are the Spices Board and the Coalition of India Industries-Food and Agriculture Center of Excellence (CII-FACE). A preliminary agreement to collaborate has already been signed by participating parties and the document is under further review by partners in India and by legal experts at the University of Maryland. A training program has been conducted in India (Phase I) in September, 2012 and planning is in advanced stages for Phase II in the spring of 2013.

International Food Safety Training Laboratory (IFSTL):

The IFSTL continues to grow and expand its course offerings. While interest in the programs is high it has been challenging for participants to secure the necessary funding. We have expanded our networking activities in an effort to secure additional funding and increase participant enrollment. Extramural funding was secured through U.S. government funding opportunities to provide both partial and full support for select participants during the current reporting period. Eight courses were delivered in 2012 (Appendix F) with a total of thirty-one participants representing government, industry and private laboratories. Early enrollment was hampered by the difficulty of potential participants in securing required funds. Countries represented in the classroom this fiscal year were: USA, Canada, Chile, China, El Salvador, Guatemala, Indonesia, Mexico, Pakistan, Peru, South Korea and Vietnam.

IFSTL actively participated in the APEC PTIN initiative, and reached out to the World Bank Global Food Safety Partnership, InterAmerican Network of Food Analysis Laboratories and many foreign government contacts. In addition, IFSTL launched several marketing initiatives including announcements in Food Safety Magazine, the American Botanical Council, American Laboratory and LinkedIn. These efforts reflect an increase in the number of countries of origin of participants and pre-registrations indicate these activities have reached the target audience.

EXPANDING GLOBAL CAPACITY BUILDING IN RISK ANALYSIS

JIFSAN's Food Safety Risk Analysis Professional Development Training Program offers a summer integrated program (SIP), residency risk analysis fellowship, customized in country training programs, and online distance learning.

The SIP program was held in June 2012 with a record number of participants (104: 29 in overview, 22 in qualitative risk assessment, 18 in risk management, 20 in risk communication, and 15 in quantitative risk assessment). Participants represented Brazil, China, Hong Kong, Italy, Japan, Malawi, New Zealand, Saudi Arabia, and Thailand. Forty-three people were trained through the on-line risk analysis program. Again, this year a risk analysis fellowship for the SIP program was offered; 12 applicants applied, Limbikani Matumba, from Malawi, received the fellowship. A Residency Fellowship (supported by ILSI China) was also offered; Xiaoyu (Joyce) Song from China was the recipient. Her research project was on Campylobacter risk assessment model for broilers.

A one day risk communication course was offered with 18 in attendance. In addition, a 4 day customized risk analysis training course was offered in Taiwan with 30 participants.

JIFSAN's Foodrisk.org, an online resource of food safety risk analysis, was revamped to highlight unique items exclusively available to the risk analysis community. Several new items have been included:

- *An Interactive online Catalogue on Risk Assessment (ICRA)* was launched in conjunction with an *ICRA* poster session at the SRA 2012 Annual Meeting on December 9th. ICRA was funded by the National Institute for Food and Agriculture (NIFA) of the United States Department of Agriculture. It is a partnership between the National Institute for Public Health and Environment (RIVM) in the Netherlands, the National Food Institute at the Technical University of Denmark, and JIFSAN. ICRA serves as a web tool offering a dynamic model catalogue for existing microbial risk assessments for risk assessors aiming to develop their own models. ICRA allows users to compare and contrast models from the same pathogen and/or commodity. The ICRA front-end, the compare and contrast models application, was built by JIFSAN IT while the back-end, data input tool, was built by RIVM. JIFSAN IT currently hosts and maintains both the front-end and the back-end on JIFSAN's local servers.

- *iRisk* was launched on Foodrisk.org October 4, 2012. *iRISK* is a free Web-based system that enables users to rank and compare risks from multiple foodborne microbial and chemical hazards and predict effectiveness of prevention and control measures. *iRisk* was launched with an hour introductory webinar watched by over 200 participants. The *iRisk* webinar recording is available on the JIFSAN website. *iRISK* was built by Risk Sciences International (RSI). RSI host and maintains the tool.

- The EPA's online *Food Commodity Intake Database* (*FCID*) was launched October 26, 2012. The *FCID's* functionality is*: (1) to improve transparency of coded fields*; *(2)* make recipes fully searchable; (3) make recipe format more user-friendly; and (4) enable users to estimate consumption of food commodities based off weighted mean and percentile calculations. The online version of *FCID* was programmed by JIFSAN IT. JIFSAN hosts and maintains this tool.

PROVIDING EDUCATIONAL RESOURCES TO HELP TRAIN A SKILLED WORKFORCE IN FOOD SAFETY AND APPLIED NUTRITION

JIFSAN offers opportunities for both students and professionals through undergraduate research internship, graduate assistantship/postdoctoral fellow, and online distance learning programs. JIFSAN education programs have provided domestic and international participants tools and techniques to assure that food products are wholesome and safe for consumption. The JIFSAN Internship program is a unique undergraduate research program designed to provide UM undergraduate students with an opportunity to collaborate with FDA scientists on specific research projects related to CFSAN's mission. The Internship Program continues to attract high caliber undergraduate students who are eager to have an opportunity to work with FDA researchers in a "hands on" environment. During the FY 2012-2013 periods, 27 students participated in the program (Appendix F).

Webinars

JIFSAN assisted CFSAN in offering two webinars in 2012: iRisk (see above) and Food and Nutrition. The **Food and Nutrition webinar (**March 5th-6th) was viewed by over 500 participants. The purpose of the webinar was to acquaint food and nutrition professionals with FDA's regulatory policies related to applied nutrition. Information provided in this web based training, assist practitioners and educators with FDA's nutrition regulatory policy issues relevant to professional practice. Twelve Continuing Professional Education Credits were given upon completion of the webinar. The webinar recordings are hosted on JIFSAN's website and have been accessed over 3,000 times since March.

Online Modules - Drug use by Foreign Producers

FDA's Center for Veterinary Medicine (CVM) has developed seven training modules on drug use by foreign producers, which were designed to clarify how the US regulates drugs for aquaculture. The modules provide information to identify what drugs may be used, explain how they should be used to prevent unacceptable residues, and identify where to look for the most current information. The content was adapted for online to be released through JIFSAN's online training portal. An alpha test was conducted in September 2012 and a beta test in November 2012 to receive feedback on module content. Comments were collected and reviewed and changes were made. The modules will be ready for release in early 2013.

Appendix A – JIFSAN Staff

Administration
Jianghong Meng, DVM, Ph.D. Director
Vernora (Nora) Petty, Assistant to the Director
Mary Grimley, Financial Officer
Pamela Biery, Business Services Specialists

International Training Program
Paul Mazzocchi, Ph.D., Associate Director
James Rushing, Ph.D., Manager, International Training Programs
Judy Cooper, Coordinator, International Training Programs
George Evancho, Senior Fellow

Risk Analysis Program
Clare Narrod, Ph.D., Manager, Risk Analysis Program

International Food Safety Training Laboratory (IFSTL)
Janie Dubois, Ph.D., Laboratory Manager
Derick Lucas, Ph.D., Chemist
Angela Winslow, Ph.D., Microbiologist
Marie Ahlgren-Stephanos, Program Management Specialist

IT Program
Kyle McKillop, Coordinator
Timothy Shaffer, IT Support Assistant
Paul Guevara, IT Support Assistant
Scott Feingold, IT Programmer

Internship Program
Kaci Thompson, Ph.D., Director of Undergraduate Research &Internship Programs, College of Computer, Math and Physical Sciences , UM

JIFSAN
STRATEGIC PLAN 2015+

I. MISSION

Founded in 1996 by the University of Maryland and US Food & Drug Administration, JIFSAN's mission is:

To advance sound strategies to improve public health, food safety, and applied nutrition using risk analysis principles through collaborative research, education, and outreach programs.

II. VISION

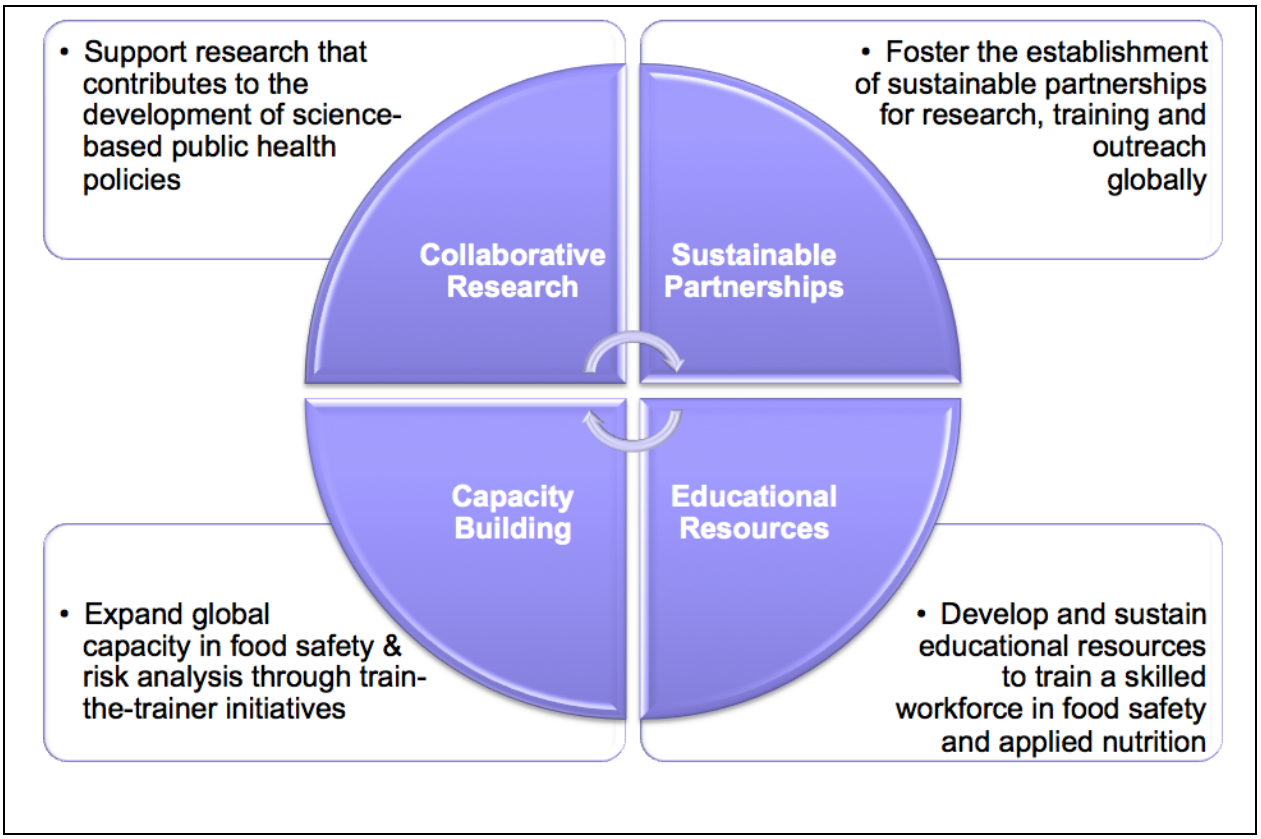
To be a premier source of scientific information and education programs on food safety and applied nutrition that enables the development of sound public health policy and reduces the incidence of food-related illness.

III. KEY OBJECTIVES

a. Increase the global knowledge of effective, available practices that promote food safety throughout the supply chain.
b. Enhance the development and promote the use of risk analysis models and tools for decision making processes associated with food safety and applied nutrition.
c. Promote collaborative research efforts related to risk analysis, food safety, and applied nutrition.
d. Broaden the research educational opportunities for undergraduate and graduate students at University of Maryland.

e. Promote the development of private and public partnerships to improve food safety.

IV. STRATEGIC THRUSTS



1. COLLABORATIVE RESEARCH

Goal	Strategies
Advance food safety and applied nutrition research that contributes to the development of science-based public health policies	1) Collaborate with FDA to conduct FDA mission-relevant research through the cooperative agreement
	2) Actively coordinate submission of proposals with various collaborators to other funding entities

	3) Leverage FDA and other supported research by developing and conducting industry-relevant research
	4) Maintain a student Internship Program a) Unique undergraduate research program designed to provide UM students the opportunity to collaborate with FDA scientists on specific research projects related to CFSAN's mission
	5) Maintain a postdoctoral fellows / visiting scholars program a) Continue to provide research scientists with opportunities to work in FDA and UM laboratories and research programs to advance new knowledge.
	6) Participate in research activities that provides international public good in food safety and applied nutrition

2. SUSTAINABLE PARTNERSHIPS

Goal	**Strategies**
Foster the establishment of sustainable partnerships for research, training and outreach globally	1) Maintain and continue to build strong partnerships with U.S. Government Agencies (e.g., FDA, USDA, etc.).
	2) Increase JIFSAN's participation in activities supported by organizations with international focus (e.g. World Bank, WTO, WHO, Codex)
	3) Increase JIFSAN's support from Industry domestically and globally

	4) Increase the number of formal agreements with local and regional organizations with compatible objectives
	5) Promote the establishment of Regional Centers for the delivery of training in food safety

3. CAPACITY BUILDING

Goal	Strategies
Expand Global Capacity in food safety and risk analysis through training initiatives	1) Continue to update Risk Analysis Curriculum a) Continue and update Summer Integrated Program (SIP) b) Expand Customized Program in Developing Countries (and or regional training programs); apply for funding to support such training through the Standards and Trade Facility; WTO, FAO c) Explore other options for online distance learning program
	2) Leverage FoodRisk.org website a) Promote www.foodrisk.org as the international source for Food Safety Risk Analysis information - Re-examine mission, expand content and improve FoodRisk.org - Provide unique online tools to users who conduct food safety risk analysis research
	3) Continue to expand the number and scope of reproducible training programs focusing on identified problems a) Develop modular programs focusing on problems (e.g. aflatoxin, pesticides, microbial contamination in produce, meats, etc.) b) Combine different delivery mechanisms (in U.S., in-country and web) to facilitate deployment at variable cost-levels
	4) Fellowship Program a) Continue (expand) Annual Tuition Fellowship SIP to recipients from Developing Countries started in 2010. a) b) Expand 3month practical training in risk

	analysis to include 2-3 students b) Expand fellowship program to other program c) d) Solicit industry support to sponsor fellowships for a specific course.

4. EDUCATIONAL RESOURCES

Goal	**Strategies**
Develop and expand the availability of educational resources for training in food safety and applied nutrition globally	1) Expand and improve current JIFSAN international training programs educational resources a) Compile training manuals (and update when needed) b) Translate to different languages c) Prepare in different formats (ppt, pdf, print)
	2) Develop educational resources for additional programs
	3) Develop distance learning resources
	4) Offer a mentoring program a) in risk analysis (SIP) b) in laboratory analysis in research
Provide a venue for information exchange in food safety and applied nutrition	5) Host relevant symposia and conferences

Appendix C – Research and Extramural Projects

COOPERATIVE PROJECTS:

Genomics of foodborne bacterial pathogens. PI: Jianghong Meng, UM, Collaborator: Eric Brown and Marc Allard, FDA/CFSAN

Comparative Genomic Analysis of *Salmonella* Newport

Salmonella Newport has emerged as a common serotype that causes human infections in the U.S. It is a polyphyletic organism and the strains within this serotype can differ significantly. A region around *mutS* was selected to determine variations between different lineages of *S*. Newport. The variation analysis would shed a light on the evolution process and genetic flux around *mutS*. The study revealed that *S*. Newport lineages II and III diverge early in the serotype evolution, and genes that delineate sublineages could be used as biomarkers for tracing back investigations during salmonellosis outbreaks.

Publication:

Cao, G., S. Zhao, E. Strain, Y. Luo, R. Timme, C. Wang, E. Brown, J. Meng, and M. Allard. 2012. Draft Genome Sequences of Eight *Salmonella* enterica Serotype Newport Strains from Diverse Hosts and Locations. J. Bacteriol. 194:5146.

Cao, G., J. Meng, E. Strain, R. Stones, J. Pettengill, S. Zhao, P. McDermott, E. Brown, M. Allard. 2012. Phylogenetics and Differentiation of *Salmonella* 1 Newport Lineages by Whole Genome Sequencing. Plos One. (Accepted)

Poster Presentation:

Cao, G., E. Strain, C. Wang, S. Zhao, E. Brown, M. Allard, and J. Meng. 2012. Comparative Genomics for *Salmonella* Pathogenicity Islands (SPI-5 and SPI-6) of *S*. Newport Using Whole Genome Sequencing. Annual Meeting of International Association for Food Protection, July 21-23, Providence, RI.

Non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC)

Shiga toxin-producing *Escherichia coli* (STEC) have been implicated in foodborne illnesses causing diarrhea and hemolytic uremic syndrome (HUS) worldwide. Although *E. coli* O157:H7 is the most important STEC serotype linked to HUS in the United States, other non-O157 STEC strains have caused HUS and outbreaks. *E. coli* O104:H4 caused a major outbreak recently in Europe (mostly in Germany) with 4,321 illnesses and an unusually high number of HUS cases (~852) including 50 deaths. The severity of the outbreak, the uncommon vehicle of transmission (seeds of fenugreek), and the unique characteristics of the outbreak strain signify the need for a thorough investigation of this serotype in the U.S. We analyzed 58 *E. coli* O104 isolates with diverse flagella (H) antigens from different animal and environmental sources. Unexpected presence of virulence markers in these isolates highlights the importance of screening unusual and potentially pathogenic Shiga toxin-producing *E. coli* serotypes.

Whole genome sequence analysis was also conducted among major non-O157 STEC strains, including O26, O111, and O103. Phylogenetic trees revealed a close relationship between O26:H11 and O111:H11 and a scattered distribution of O111. The study indicated that STEC serotypes with the same H antigens might share common ancestors. The data can lead to the development of diagnostic tools for identifying high risk STEC.

Publication:

Rump, L.V., S. Bodies-Jones, J. Abbott, S. Zhao, J. Kase, S. Lorenz, M. Fischer, E. Brown, and J. Meng. 2012. Genetic characterization of *Escherichia coli* O104 isolates from different sources in the United States. Appl. Environ. Microbil. 78: 1709–1717.

Ju, W., G. Cao, L. Rump, E. Strain, Y. Luo, R. Timme, M. Allard, S. Zhao, E. Brown, and J. Meng. 2012. Phylogenetic analysis of non-O157 Shiga toxin-producing *ESCHERICHIA COLI* by whole genome sequencing. J. Clin. Microbiol. 50: 4123-4127.

Poster Presentation:

Ju, W., S. Shen, M. Toro, S. Zhao and J. Meng. 2012. Distribution of pathogenicity islands in Shiga toxin-producing *Escherichia coli.* Annual Meeting of International Association for Food Protection, July 21-23, Providence, RI.

Metrics for evaluating effectiveness of international food safety trainings PI: Clare Narrod (UM)

Since 2000, JIFSAN has been developing and conducting training programs for the global food safety community. The goal of these programs is to increase the knowledge and use of best practices that improve the quality and safety of food through “Train-the-Trainer” programs. The Food Safety Modernization Act (FSMA) aims to ensure the U.S. food supply is safe by shifting focus of federal regulators from responding to contamination to preventing it. This change places a stronger emphasis on monitoring the effectiveness of these programs. JIFSAN researchers have developed a set of public health metrics tools to evaluate individual participants knowledge prior to and immediately upon completion of the training programs. These tools were piloted during recent training courses and preliminary results indicate a high level of knowledge transfer. The immediate goal is to continue to refine these tools in an effort to provide JIFSAN with accurate measurements to assess the effectiveness of its training programs.

Development and Validation of Isotope Methods for Distinguishing Between Naturally Occurring and Synthetic Phthalates in Food PI: John Ondov, UM, Collaborator: Michael VanDerveer, FDA/CFSAN

“Phthalates” (1,2-benzenedicarboxylic acid esters, herein PAE) are used as plasticizers (compounds used to make plastics more flexible), as a solvent for oil-soluble dyes and nitrocellulose lacquers, as an antifoaming agent, a fiber lubricant in the textile industry, a fragrance fixative, and an alcohol denaturant. As such they are used in a variety of consumer products including inks used to print labels on food packaging. The developed methods could be implemented by FDA laboratories in detecting synthetic phthalates in food for regulatory purposes.

Presentations:

1) Nelson, M. A, Ondov, J. M.,, "Contemporary Fraction of bis(2-ethylhexyl) Phthalate in Stilton Cheese and Butter by Accelerator Mass Spectrometry" at the 2nd World Congress on Analytical and Bioanalytical Techniques, December 16-17, San Francisco, USA.
2) Nelson, M. A., Ondov, J. M., VanDerveer, M. C., Buchholz, B., "Contemporary Fraction of bis(2-ethylhexyl) Phthalate in Stilton Cheese and Butter by Accelerator Mass Spectrometry: Preliminary Results," abstract accepted for presentation at Radiocarbon 2012, July 9-13, Paris, France.
3) Ondov, J. M., Nelson, M. A., Tong, T., "Contemporary DEHP Carbon Fraction in Stilton Cheese Sample ST01: Results and Error Analysis" briefing presented to FDA, February 23, 2012.

EXTRAMURAL PROJECTS

Food Safety Training Programs to Cochran Fellows – Funded by USDA-FAS. PI: Janie Dubois (JIFSAN, UM); $24,000

The IFSTL hosted 8 Cochran Fellows in 2012. The Cochran Fellowship Program provides participants from middle-income countries, emerging markets, and emerging democracies with high-quality training to improve their local agricultural systems and strengthen and enhance trade links with the United States. Participants are mid- and senior-level professionals from both the public and private sectors who are concerned with agricultural trade, agribusiness development, management, policy, and marketing.

Indonesia (1): Laboratory training in methods for the detection of *E. coli* and *Salmonella*.

Guatemala (4): Laboratory training in methods for the determination of mycotoxins (3) and the detection of *E. coli* (1).

El Salvadore: Laboratory training in the detection of *Cronobacter* in infant formula.

Thailand (2): Laboratory training in methods of determination of pesticide residues and introduction to ISO 17025.

APEC Laboratory Capacity Building – Methods in Pesticide Residue Analysis – Funded by USDA – FAS. PI: Janie Dubois (JIFSAN, UM); $131,740

In FY 2012, this Emerging Market Program project was executed to provide laboratory training for laboratory scientists who can then become trainers in their country in operational and testing methods to meet U.S. and international standards (train-the-trainer). Proficiency in laboratory analyses is vital to maintaining a functional food safety system, meeting the requirements for most export markets, and ensuring the application of internationally recognized analytical methods and tests. Laboratory capacity building had been deemed a priority by the APEC/PTIN

Expert Working Group in 2010 to address the global context of food safety. The “train-the-trainer” approach enables the efficient delivery of training to a broader group of laboratory workers than would be able to be trained either abroad or in country. In addition, the use of IFSTL is a means of leveraging private and federal funding to meet a common objective.

The project is focused on developing and delivering two forms of training on the specific topic of pesticide residue analysis in fresh fruits and produce, with the intent to address a series of other laboratory analytical methods critical for food safety and commercial trade over the next three to five years.

The activities undertaken in this project were:

Hands-on laboratory training in methods of pesticide residue analysis
Development and delivery of 5 online modules on pesticide residue analysis

The hands-on laboratory training took place during the week of May 14-18, 2012. Twelve qualified participants from emerging economies in the APEC (Chile, Indonesia, Mexico, China, Philippines, Peru and Vietnam) and one participant from a non-emerging country (Canada) traveled to the University of Maryland. The 5-day workshop included lectures from U.S. FDA (CFSAN), EPA and University of Maryland subject-matter experts and laboratory work including sample preparation, standards preparation, analysis, data analysis and verification. The train-the-trainer program included a heavy load of hands-on laboratory work performed on green peppers, oranges and ginseng. The workshop trained participants in building and using multi-residue methods, using a 200-residue dataset for the exercise, following the QuEChERS sample preparation method.

Five online training modules were also prepared, which consist in narrated presentations and learning evaluations through multiple-choice questions integrated in the modules. The modules start with an introduction to pesticide residue analysis, followed by technical modules on regulatory concepts, sample preparation and chromatography, mass spectrometry and method validation. The test questions include short case studies that require the use of the resources described in the modules in addition to the theoretical knowledge presented. The modules will be available on the APEC PTIN website (which is administered by JIFSAN).

Appendix D - Publications

Research Articles:

1) Xia, X., Y. Luo, Y. Yang, K. Schneider, and J. Meng. 2012. Effects of Tomato Variety, Temperature Differential and Post-stem Removal Time on Internalization of *Salmonella Enterica* Serovar Thompson Into Tomatoes. J Food Prot. 75:297-303.

2) Rump, L.V., S. Bodies-Jones, J. Abbott, S. Zhao, J. Kase, S. Lorenz, M. Fischer, E. Brown, and J. Meng. 2012. Genetic characterization of *Escherichia coli* O104 isolates from different sources in the United States. Appl. Environ. Microbil. 78: 1709–1717.

3) Rump, L.V., J. Meng, E. A. Strain, G. Cao, M.W. Allard, and N. Gonzalez-Escalona. 2012. Complete DNA sequence analysis of enterohemorrhagic *Escherichia coli* plasmid pO157_2 in β-glucuronidase-positive *E. coli* O157:H7 reveals a novel evolutionary path. J. Bacteriol. 194:3457-63.

4) Ju, W, J. Shen, Y. Li, M.A. Toro, S. Zhao, S. Ayers, M.B. Najjar, and J. Meng. 2012. Non-O157 Shiga toxin-producing *Escherichia coli* in retail ground beef and pork in Washington DC area. Food Microbiol. 32:371-377.

5) Cao, G., S. Zhao, E. Strain, Y. Luo, R. Timme, C. Wang, E. Brown, J. Meng, and M. Allard. 2012. Draft Genome Sequences of Eight *Salmonella* enterica Serotype Newport Strains from Diverse Hosts and Locations. J. Bacteriol. 194:5146.

6) Ju, W., G. Cao, L. Rump, E. Strain, Y. Luo, R. Timme, M. Allard, S. Zhao, E. Brown, and J. Meng. 2012. Phylogenetic analysis of non-O157 Shiga toxin-producing ESCHERICHIA COLI by whole genome sequencing. J. Clin. Microbiol. 50: 4123-4127.

7) Subramanian, S., K. Aschenbach, J. P. Evangelista, M. B. Najjar, W. Song, R. D. Gomez. 2012. Rapid, sensitive and label-free detection of Shiga-toxin producing Escherichia coil 0157 using carbon nanotube biosensors. Biosensors & Bioelectronics. 32:69-75.

8) Micallef, S.A. , R. E. Rosenberg Goldstein, A.H. George, L. Kleinfelter, M. S. Boyer, Cr. R. McLaughlin, A. Estrin, L. Ewing, J. J. Beaubrun, D. E. Hanes, M. H. Kothary, B. D. Tall, J. H. Razeq, S. W. Joseph, A. R. Sapkota. 2012. Occurrence and antibiotic resistance of multiple Salmonella serotypes recovered from water, sediment and soil on mid-Atlantic tomato farms. Environmental Research. 114:31-39.

Appendix E - International Trainings

Good Agricultural Practices (GAP):

December 6-9, 2011, Mexico City, Mexico, in collaboration with US-FDA, SENASICA-SAGARPA and Calidad Suprema. There were 36 participants formally enrolled in the training but 81 were present for the opening session. Attendees included managers from the private sector and representatives of government and academia.

June 11-14, 2012, Tela, Honduras, in collaboration with Servicio Nacional Sanidad Agropecuaria (SENASA-Honduras) and the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA). This was a regional program with 66 people enrolled, twenty-four of whom were trainers from the region as follows: Costa Rica (1), Belize (2); El Salvador (2); Guatemala (2); Honduras (6); Mexico (2); Nicaragua (2); Panamá (3), Dominican Republic (2), and the OIRSA office (2). The remaining forty-two participants were from the private sector.

Supply Chain Management for Spices and Botanical Ingredients

September 17-21, 2012 Kochi (or Cochin), Kerala, India, a new JIFSAN international training program was planned and delivered in collaboration with the Spices Board and the Coalition of India Industry-Food and Agriculture Center of Excellence (CII-FACE). Seventy three participants were present on the opening day.

Good Aquacultural Practices (GAqP):

December 3 – 7, 2011, Khulna, Bangladesh in collaboration with Bangladesh Shrimp and Fish Foundation with supporting in-country sponsorship from Bangladesh Aquaculture Alliance, Trade Services International, Ltd., Bangladesh Department of Fisheries, Ministry of Fisheries & Livestock, and the Bangladesh Department of Commerce. This program, with over 20 participants, was a continuation of activity conducted by the collaborative Aquatic and Aquaculture Food Safety Center (AAFSC) established in 2009 by JIFSAN and its partners as described previously. Five Bangladeshi instructors presented CFSAN-JIFSAN pre-approved GAqP materials which they themselves had been trained on the previous year. Supplemental instruction was offered by JIFSAN and FDA instructors.

January 17-21, 2012, Chennai, India, held at the Madras Veterinary College in collaboration with the Coastal Aquaculture Authority (CAA) and the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). There were 38 participants from the Indian government (19), academia (10), and industry (9). Government participants represented the Costal Aquaculture Authority, the Marine Products Export Development Authority, the Export Import Agency, and the Department of Fisheries.

December 3 – 7, 2012, Can Tho, Vietnam in collaboration with the National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD). NAFIQAD provided in-country support as well as the American Soybean Association sponsoring simultaneous translation services. This program was offered to 32 participants. Fourteen individuals participated in an online and one-day in-classroom HACCP certification training.

Food Inspector Training (FIT)

May 21 – 30, 2012, Nanning, Guangxi, China in collaboration with the US-FDA and the China Inspection and Quarantine Association (CIQA) based in Beijing, China. The pilot program was offered to 75 participants, approximately 35 of whom were professional food inspectors who participated in several extra days of training that involved mock inspections at two food processing facilities

Appendix F - IFSTL Courses

Microscopic Identification of Botanicals (December 2011): Six participants from the U.S. dietary supplement industry came to the IFSTL for a 3-day workshop. Two FDA and one FDA-retired instructors guided the participants through hands-on work that led to each presenting a short monograph of a plant material we had obtained from the University of Mississippi for this course.

Methods of Detection of Mycotoxins (April 2012): Six participants originating from government laboratories in Guatemala, South Korea and Indonesia participated in this 8-day workshop. The instruction was led by FDA-retired Mary Trusksess and involved lectures by subject-matter experts from USDA (2), FDA (5) and the University of Maryland (3).

Methods of Detection of Pesticide Residues in Fruits and Vegetable –Focus on APEC (May 2012): This course received financial support from the USDA Emerging Markets Program, which provided travel funds for over half the participants and registration fees for all participants from developing countries. The class was made up of one participant from a private laboratory doing government contract work in Canada, analysts from government laboratories in Chile, Indonesia, Mexico and China, a research team from academia in Vietnam and agricultural trade specialists from Peru and Chile. The 5-day workshop included lectures from U.S. FDA (CFSAN), EPA and University of Maryland subject-matter experts and laboratory work including sample preparation, standards preparation, analysis, data analysis and verification. The train-the-trainer program included a heavy load of hands-on laboratory work performed on green peppers, oranges and ginseng.

Detection of *Cronobacter* in Infant Formula (June 2012): Three participants from the U.S infant formula industry, one participant from a national laboratory in El Salvador and two PhD students from the University of Maryland took this class, where one of the students also acted as translator for the participant from El Salvador). This was the first activity of our microbiology curriculum and it involved 5 instructors from the FDA.

Methods of Detection of Salmonella in Fresh Produce (September 2012): This course was not as popular as expected. A government researcher from Pakistan, and a provincial government laboratory analyst from Indonesia registered, and we offered complimentary registrations to a local unemployed microbiologist and a PhD student in food microbiology at the University of Maryland.

Determination of enterhemorragic *E. coli* (EHEC) in Fresh Produce and Meat (October 2012): The course was designed for laboratory scientists and technicians involved in testing fresh produce or meat for the presence of EHEC. Seven professionals from Korea, Indonesia, and Guatemala participated in the training course.

Dietary Supplements: Microscopic and Chemical Identification of Botanicals (October 2012): The course focuses on the confirmation of identity of plant materials used in dietary supplements. Six US participants took the course.

Methods of Pesticide Residue Analysis and laboratory accreditation to ISO 17025 (November, 2012): This course teaches laboratory scientists and managers involved in the use or development of GC and LC methods for the detection and quantitation of pesticide residues in fresh fruits, vegetables and processed foods. One day of this course was

dedicated to the requirements for accreditation to ISO/IEC 17025. Ten people from Dominican Republic, Mexico, Korea, Thailand, US, and Malaysia participated in the training.

2013 course schedule is available online: http://ifstl.org/courses/

Appendix G – Undergraduate Internship/Graduate Assistantship

Undergraduate Internships (FY 2012)				
Last Name	First Name	Project ID	Project	Mentor
Blazar	Jeffrey	JIP-194	Characterization of Salmonella enterica subsp. enterica serovar Newport isolates Associated with the Outbreak of 2010.	Marc Allard
Chou	Luoth	JIP-219	Adaptation of Listeria monocytogenes in high osmolarity and refrigeration temperature.	Atin Datta
Dickey	Erin	JIP-220	Development of a method for the detection and isolation of Salmonella from internally and externally contaminated tomatoes	Thomas Hammack
Do	Andrew	JIP-218	To identify allergenic proteins of the major food allergens and to determine their digestibility and IgE immunoreactivity.	Ondulla Foye-Jackson
Gathercole	Laura	JIP-147	Food Safety Risk Analysis: Quantitative Risk Assessments	Sherri Dennis/Yuhuan Chen
Islam	Emrul	JIP-205	Characterization of Housekeeping Genes in Salmonella enterica for their phylogenetic accuracy and SNP discovery	Rebecca Bell
Joo	Jane	JIP-211	Cyclospora cayetanensis and Cryptosporidium parvum: Methods Development	Joan Shields
Kuo	Jennifer	JIP-210	Establish methodology for assessing inflammatory cytokine expression (mRNA and protein) in infectious and inflammatory models of foodborne pathogens.	Mohammad Samiul Alam
Lee	Chloe	JIP-217	Development of a multiplex real-time PCR method for simultaneous detection of Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes in soft cheese and spinach	V. Sathyamoorthy
Lee	Hannah	JIP-216	Determine the bacterial load of food borne pathogens carried by flies	Monica Pava-Ripoll
Lee	Nathan	JIP-215	Analysis of Chemical Contaminants in Foods	Jon Wong
Mehta	Akshita	JIP-213	Assessment of pathology and immunological biomarker expression in mice following exposure to STEC-associated Shiga Toxin 2.	Lisa Plemmons

Niazi	Nicholas	JIP-222	Fungal and aflatoxin contamination of milk thistle supplements	Valerie Tournas
Patel	Ronak	JIP-212	To identify allergenic proteins of the major food allergens and to determine their digestibility and IgE immunoreactivity.	Ondulla Foye-Jackson
Shyong	Nicole	JIP-223	Food Irradiation: Chemical Changes in Food and Food Contact Substances due to the Absorption of Ionizing Radiation	Kim Morehouse
Tangri	Vidur	JIP-176	Bioinformatic annotation of detection and food outbreaks literature related to C. botulinum in foods	Shashi Sharma
Thole	Joseph	JIP-221	Rapid Methods for the Detection of Food Allergens and Toxins	Eric Garber
Vanegas	Camilo	JIP-198	Study of shellfish toxicity off the coast of New England	Stacey DeGrasse
Undergraduate Internships (FY 2013)				
Anders	Stephen	JIP-223	Food Irradiation: Chemical Changes in Food and Food Contact Substances due to the Absorption of Ionizing Radiation	Kim Morehouse
Chou	Luoth	JIP-219	Adaptation of Listeria monocytogenes in high osmolarity and refrigeration temperature.	Atin Datta
Do	Andrew	JIP-218	To identify allergenic proteins of the major food allergens and to determine their digestibility and IgE immunoreactivity.	Ondulla Foye-Jackson
Hahn	Justin	JIP-226	Identification of virulence factors that contribute to the enterotoxicity of Vibrio parahaemolyticus.	Augusto Franco-Mora
Kanyuck	Kelsey	JIP-225	Method Validation for Seafood Toxin Biosensors	Betsy Yakes
Kuo	Jennifer	JIP-210	Establish methodology for assessing inflammatory cytokine expression (mRNA and protein) in infectious and inflammatory models of foodborne pathogens.	Mohammad Samiul Alam
Lee	Nathaniel	JIP-215	Analysis of Chemical Contaminants in Foods	Jon Wong
Lopez	Joanna	JIP-147	Food Safety Risk Analysis: Quantitative Risk Assessments	Yuhuan Chen/Sheri Dennis
Meltsner	Juliet	JIP-213	Assessment of pathology and immunological biomarker expression in mice following exposure to STEC-associated Shiga Toxin 2.	Lisa Plemons

Moore	Amanda	JIP-228	Use and Applicability of Human Clinical Studies in the Generally Recognized As Safe (GRAS) Program	Timothy Twaroski
Park	Joseph	JIP-224	Analyzing Food Safety Practices Related to Fresh Produce	Joy Johanson
Saunders	Cameron	JIP-227	Isolation and identification of yeasts with antagonistic activities against Penicillium expansum, the main cause of postharvest spoilage and patulin production in apples	Valerie Tournas
Thole	Joseph	JIP-221	Rapid Methods for the Detection of Food Allergens and Toxins	Eric Garber
Trach	Larisa	JIP-217	Development of a multiplex real-time PCR method for simultaneous detection of Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes in soft cheese and spinach	Venegupal Sathuanoorthy
Yang	Kwon	JIP-147	Food Safety Risk Analysis: Quantitative Risk Assessments	Jane Van Doren/Sheri Dennis
Graduate Assistantship				
Li	Jinxi		Development of Methods for the Characterization of Protein Allergens	John Callahan (CFSAN) / Catherine Fenselau (UM)
Cao	Guojie		Whole Genome Sequencing of Salmonella Newport	Marc Allard (CFSAN) / J. Meng (UM)

Appendix H – Symposia/Conferences

Disease Outbreak Detection in the Genomics Era: Global Road Map Meeting #2 (March 1st and 2nd, 2012, Arlington, Virginia). The meeting was (i) To provide an expanded follow-up to the 2011 Brussels meeting and debate the short and long term obstacles and solutions for a global system for identification of microorganisms based on genomic information. (ii) To provide an overview of ongoing initiatives and discuss how global collaboration can be achieved. (iii) To determine a path forward for how to establish a globally distributed system. A series of draft road map statements were developed and they focused on establishing a global disease outbreak detection system using a worldwide network of shared genomic information for bacterial, viral, and parasitic microorganisms and a commitment from various institutions for working together on developing such a database. Agilent Technologies, Inc. announced a public-private partnership to sequence 100K genomes of foodborne pathogens (http://100kgenome.vetmed.ucdavis.edu/about/index.cfm).

APEC Export Certification Workshop, held April 24-25, 2012 in Greenbelt, Maryland. The Asia- Pacific Economic Cooperation (APEC) region accounts for nearly 60 percent of world trade and U.S. agricultural exports. Misunderstandings over export certification, different interpretations of Codex standards, and varying country-by-country requirements create barriers to U.S. exports and increase the costs of doing business to U.S. exporters in many of the APEC countries. The disputes over export certificates cover many different commodities from fresh meat and live cattle to milk and processed products. An initial workshop, held February 2010, under the auspices of the APEC Food Safety Cooperation Forum, allowed APEC country representatives to discuss the range of issues that negatively affect U.S. and other APEC exports and developed several action items to streamline the certification process and reduce future restrictions.

Annual Advisory Council Spring Symposium, ***Feeding the World Population Today and Tomorrow***, held May 16-17, 2012 in Greenbelt, Maryland. According to current projections, the global population will reach eight billion by 2025-2030, and will likely reach nine billion by 2045-2050. Feeding this expanding population and meeting rising consumer expectations present production, nutritional and food safety challenges that will require new approaches and unique solutions. Local issues can quickly become global issues with the continuing globalization of the food supply. Experts from the government, academia, and the industry offered their insights on these issues under three sessions: Global Food Safety Initiatives, Turning the Page from "Chemo and Microphobia" to "Food Safety", and Role of Processed Foods in Nutrition and Disease. Over 70 participants attended the symposium. More information is available at http://jifsan.umd.edu/events/event_record.php?id=62

13th Fera/JIFSAN Symposium (June 13 – 15, 2012, York, UK). The thirteenth in a series of annual symposia in food science jointly organized with the Food and Environment Research Agency (Fera), UK, focused on "New developments in food science: realizing the potential of 'omics' technologies". Genomic, proteomic and metabolomic technologies are rapidly developing, enabling capabilities (or resources) in biological sciences that will have far reaching impacts in the food and agricultural science sectors. The Symposium aimed to explore the potential applications of these technologies in a variety of areas relating to food and sustainable

crop production. It brought together industrial, academic and government scientists to explore the present and future impact of ‘omics’ technologies in food science. The discussions indicated that the profound changes that ‘omics’ technologies could deliver in their respective sectors.