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The National Molecular Subtyping Network
for Foodborne Disease Surveillance



PulseNetTM News

State & Local Public Health Laboratories
in the United States and PulseNet Canada



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OUTREACH TO CLINICAL LABORATORIES SUPPORTS PULSENET

Patricia A. Somsel, Dr.P.H., Director, Division of Infectious Diseases, Bureau of Laboratories, Michigan Department of Community Health, Lansing, MI

Like most state laboratories, the Michigan Department of Community Health (MDCH) Bureau of Laboratories (BOL) depends upon clinical laboratory submission of isolates that have been recovered from patient specimens. We have sought to strengthen the ties to our clinical microbiology laboratories because of this dependence and for several additional reasons. First, of course, we needed to ensure that clinical laboratories were aware of the value of submitting their isolates for our surveillance efforts. Simple reporting of the recovery of *Salmonella*, *Shigella*, STX-producing *E. coli*, and *Listeria*, for instance, is not sufficient for current public health purposes. Yet we found most clinical microbiologists were unaware of the way submitted organisms were being used. PulseNet was not a word which held any meaning for the overwhelming majority of clinical microbiologists in Michigan.

As a first step, in the mid-to-late 1990's, we compiled a database of all clinical microbiology laboratories in the state by searching Clinical Laboratory Improvement Act (CLIA) program records and sorting by specialty.

We then sent a letter to all clinical microbiology laboratory supervisors/directors requesting submission of all isolates of *Listeria monocytogenes*



to MDCH BOL. *Listeria* was an organism which we had not previously requested be submitted to the BOL. Although compliance was not complete, it was sufficient enough to provide support for the investigation of the outbreak in 1998-99 of listeriosis associated with deli turkey and hot dogs from a processor located in Michigan. Because the outbreak received a great deal of press coverage, the value of submission to the MDCH BOL was reinforced.

In the ensuing years, we have used our laboratory newsletter, *LabLink*, and the Michigan Society for Infection Control Listserv to promote submissions. We published an article in the former, detailing the investigation of two outbreaks, and the importance of **(Continued on page 2)**

PLANNING MEETING FOR DNA SEQUENCING-BASED SUBTYPING

Patricia I. Fields, PhD, Chief, National Salmonella and Campylobacter Reference Laboratories, Centers for Disease Control and Prevention, Atlanta, GA

The PulseNet Planning Meeting for DNA Sequencing-based Subtyping was held in San Antonio on Friday May 2, in conjunction with the 7th Annual PulseNet Update Meeting. The purpose of the meeting was to discuss progress towards the development of the "next-generation" subtyping methods that can be employed by PulseNet laboratories to complement or replace existing methods for bacterial subtyping. While pulsed-field gel electrophoresis (PFGE) analysis is currently "state of the art," lab-to-lab comparisons of data require

strict adherence to labor-intensive standardized PFGE protocols in order to obtain comparable results. DNA sequencing-based approaches to bacterial typing have almost no experimental variation in results, allowing direct inter-laboratory comparisons, and offer more precise information on strain relatedness than does PFGE. With rapidly improving methods for and declining costs of DNA sequencing technology, we hope to be able to simplify subtype data production and analysis, improve strain discrimination, **(Continued on page 2)**

submissions from clinical microbiology laboratories to the PulseNet system which helped identify the source of the infections. One of these involved a *Salmonella* ser. Infantis associated with hatchling fowl in Michigan, which was of local interest. We rewrote the article for hospital infection control practitioners to underscore the importance of reporting communicable diseases so that sources of disease could be identified, and to encourage clinical microbiology laboratories submissions.

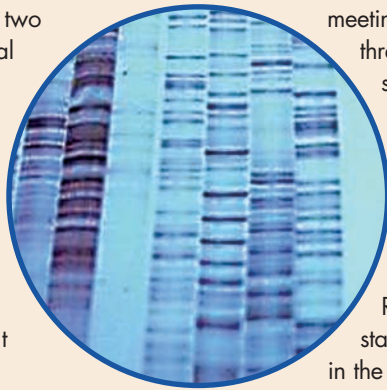
The second reason we chose to enhance our clinical laboratories ties was the realization that the knowledge base and capability of clinical laboratories in the area of microbiology are under assault. Laboratory training programs have been shrinking; we are producing only about half of the nearly 9,000 laboratory professionals needed annually. With the average age of clinical laboratory scientists approaching fifty, and insufficient graduates to replace those leaving the field every year, clinical labo-

raries have employed varying methods to deal with vacancies. According to the Lewin workforce study commissioned by the American Society for Microbiology (ASM) in 1998, the vacancy rates have reached 20% in some microbiology laboratories. Many have recruited associate and baccalaureate level chemists and biologists and provided on-job-training. While training programs have stepped up to the challenge and broadened the training of the technician programs, **(Continued on page 5)**

SEQUENCING-BASED SUBTYPING *Continued from the cover*

and reduce response time for outbreak investigation and other public health questions.

The DNA sequencing-based subtyping project was initiated two years ago, at the 5th Annual PulseNet Update Meeting, with the announcement of a Request for Proposals (RFP), funded by Association of Public Health Laboratories (APHL) and CDC, to support research in three public health laboratories aimed at the development of DNA sequencing based subtyping methods for *E. coli* O157:H7; *Salmonella* spp., and *Listeria monocytogenes*. At that time, Dr. Andy Sails at CDC had initiated a program to develop DNA sequencing based subtyping methods for *Campylobacter* based on multilocus sequence typing (MLST) as a "proof-of-concept." Thirteen proposals were received and reviewed. Grants were awarded to two state health laboratories in the fall of 2001: to Massachusetts to develop methods for *E. coli* O157:H7 and to Minnesota to develop methods for *Salmonella* spp. After a second round of RFPs, a grant was awarded to North Carolina to develop methods for *L. monocytogenes* in January 2003. The three principal investigators on the projects are Dr. Sandy Smole from Massachusetts, Dr. Kristin Pederson from Minnesota, and Dr. Leslie Wolf from North Carolina. The three groups are employing a combination of comparative DNA sequencing and variable number tandem repeat (VNTR) analysis in their



development of improved methods for subtyping these organisms.

At the last session of the update meeting on Friday morning, the three principal investigators presented overviews of their research. The PulseNet Planning Meeting for DNA Sequencing-based Subtyping was convened Friday afternoon after the close of the main meeting. Representatives from the three state health laboratories involved in the project, CDC, Health Canada, PulseNet Europe, and PulseNet Asia Pacific attended the meeting. Also in attendance were a panel of "outside experts," including Drs. Sophia Kathariou, Tom Whittam, and Andy Sails, whose role was to critically evaluate our program and offer input and suggestions for experimental protocol and research direction.

Dr. Paul Vauterin from Applied Maths began the DNA sequence-based subtyping meeting with a discussion of features/enhancements for BioNumerics that can be applied to the analysis of DNA sequence data and variable number tandem repeats (VNTRs), two approaches that have been employed in the development of DNA sequence-based subtyping methods. A VNTR is a defined region of DNA, typically a non-coding region that contains multiple copies of a short sequence of nucleotides. Individual isolates often have different numbers of copies of the repeated sequence at a particular VNTR locus. Differences in copy number are detected as

differences in size of a PCR fragment that encompasses the VNTR locus, typically determined by analyzing the PCR fragments on a DNA sequencer. Multilocus VNTR analysis (MLVA) is the analysis of multiple VNTR loci for the purpose of subtyping; this approach has been quite successful in differentiating very clonal organisms such as *Bacillus anthracis* and *Mycobacterium tuberculosis*.

Next, each of the principal investigators from the participating public health departments gave an in-depth discussion of their research program, followed by a discussion of the development of a DNA sequencing-based subtyping method for *Campylobacter* based on MLST by Andy Sails. Sandy Smole and Kristin Pederson have both employed VNTR analysis for DNA sequence-based subtyping of *E. coli* O157:H7 and *Salmonella* spp., respectively. Both groups are having good success differentiating isolates, with the possible exception of *S. Enteritidis*, using VNTR analysis. We are hopeful that a combination of comparative DNA sequencing and VNTR analysis will prove to be fast and simple, and facilitate inter-laboratory comparison of data between PulseNet laboratories.

This was the first time that a planning meeting for DNA sequencing-based subtyping was held in conjunction with the PulseNet Update Meeting, and this format was deemed a success. We plan to continue to convene the two meetings together which should allow greater participation of other PulseNet laboratories. Also, look for training related to this new approach to subtyping at future PulseNet and BioNumerics workshops. **CDC**

North Carolina Salmonella Enteritidis Outbreaks, 2001

Leslie A. Wolf, PhD, Assistant Laboratory Director, and Denise G. Briggs, MT (ASCP), Laboratory Medical Specialist, North Carolina State Laboratory of Public Health, Raleigh, NC

During 2001, North Carolina experienced a large increase in the number of human infections caused by *Salmonella* Enteritidis. There were 71 cases in 1999, 90 cases in 2000, and 222 cases in 2001. A comparison of the number of *S. Enteritidis* isolates from the first six months of years 2000 and 2001 illustrates the increase observed in North Carolina in 2001 (Table 1).

Half of the isolates collected during March 2001 originated from three counties; including Columbus, Forsyth, and Mecklenburg. At the request of the PulseNet Task Force at CDC, we performed PFGE subtyping on 16 of 18 isolates collected (2 isolates were unrecoverable) (Figure A). Eight isolates were designated NC pattern D (PulseNet pattern JEGX01.0021), which also was associated with an outbreak in February 2001 in South Carolina (MMWR 51(51); 1149-1152). The PulseNet national database indicated this is a less commonly reported pattern. Three isolates were designated NC pattern B (PulseNet pattern JEGX01.0004), the most common pattern in the PulseNet database for *S. Enteritidis*.

A restaurant-associated outbreak occurred in June 2001 in Mecklenburg County. PFGE analysis was performed on 13 of 31 isolates collected in June. Eight of 13 isolates were NC pattern B, and 2 were NC pattern D. From this and the previous outbreak period, 6 isolates with NC pattern D and 6 isolates with NC pattern B, were subtyped using a second

Month	2000	2001
January	1	12
February	4	11
March	2	18
April	5	19
May	6	13
June	23	31

North Carolina *S. Enteritidis* isolates in first 6 months of 2000 and 2001.

enzyme *AvrII*. *AvrII* restriction however, did not show further discrimination within these two pattern types.

Another peak in cases occurred in July 2001; 48 cases of *S. Enteritidis* were identified (Figure B). When the large number of cases became apparent, a team of epidemiologists from CDC was invited to Raleigh to work with state epidemiologists and local health departments to determine the source of the outbreak. PFGE analysis was performed on 118 isolates collected from July 2001 to the end of the year. Analysis showed that 26 of 48 isolates from July 2001 were NC pattern D, 8 of 48 were NC pattern B, and 5 of 48 were NC pattern C (PulseNet pattern JEGX01.0005). On the basis of information gathered by the epidemiologists from patient interviews and the PFGE data, a strong association emerged between patients who had PFGE pattern D and egg consumption. Of the 46 NC pattern D isolates, 14 were phage typed, 13 were phage type 13a and 1 had a reaction but did not conform. Phage type 13a was previously linked to an egg distributor in South Carolina whose product was implicated in the February 2001 outbreak of *S. Enteritidis* in that state (MMWR 51(51); 1149-1152). As a result of the data gathered from this outbreak, an FDA traceback investigation was initiated. The FDA traceback was inconclusive, however, and only egg consumption, PFGE pattern, and phage type linked this large cluster of cases to each other.

Although *S. Enteritidis* is highly clonal with a high background level of cases each year, PFGE was useful in separating outbreak cases from non-outbreak cases. Clearly, egg safety issues remain in our state and must be addressed "from the farm to the fork." **CDC**

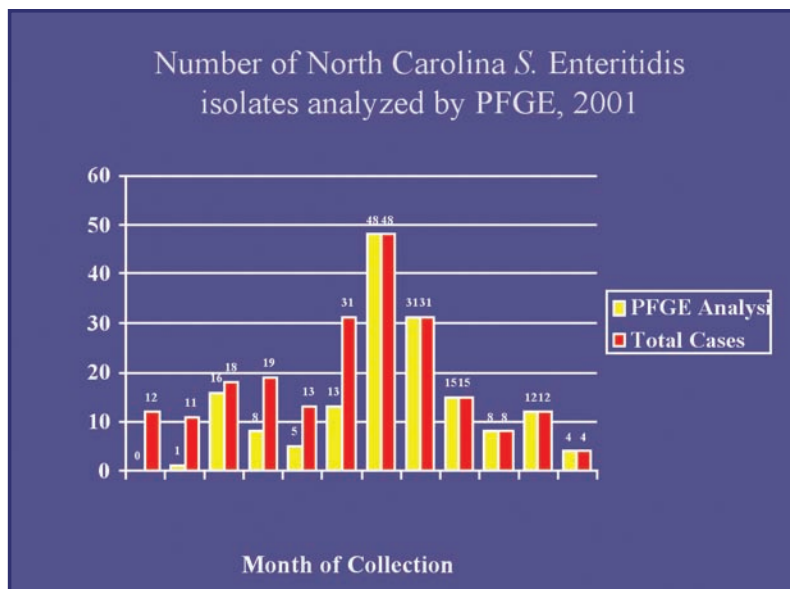
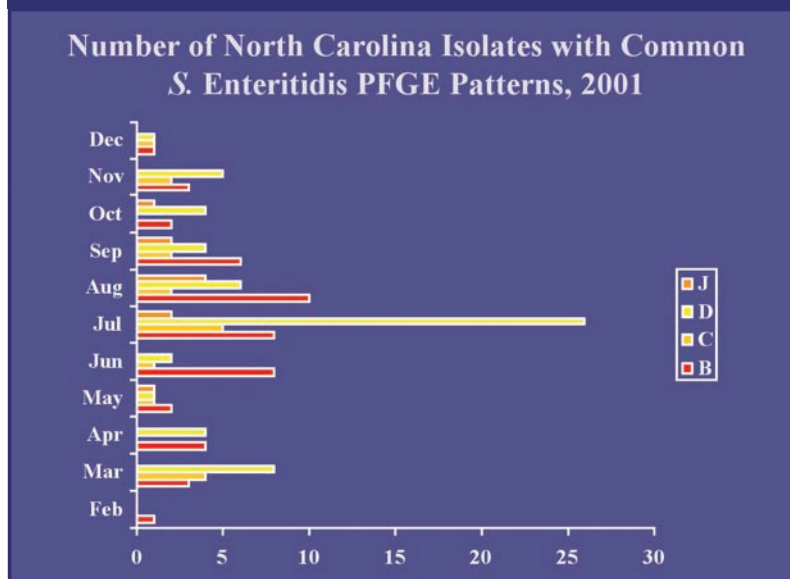


Figure A (above) and Figure B (below)



Q&A: BIO NUMERICS

The questions in this section were gathered during the breakout sessions at the 2003 PulseNet Update Meeting. If you have a question you would like to submit for future issues of the PulseNet Newsletter, please send an email with the subject heading 'Newsletter' to: PFGE@cdc.gov.

Q. Some files were not converted during the database conversions from Molecular Analyst to BioNumerics. Is it important to 'fix' this?

A. It is important to reanalyze any TIFF in BioNumerics that did not convert properly. There may have been a corrupt file associated with the MA-F (Gel Compar) analysis of this TIFF which prevented conversion. Look for unlinked isolates in your BioNumerics databases. Typically, you will not know that a file did not convert correctly until you search for one that isn't present in your BioNumerics database. If you do come across a group of TIFFs that did not convert properly, we suggest reanalyzing the most recent ones first and work backwards.

Q. If a state runs many gels of isolates with the same PFGE pattern during an outbreak, is it necessary to submit each TIFF, or is it sufficient to report the number of PFGE-indistinguishable cases and provide any other specific required descriptive information?

A. It is important to submit all isolates to the online database or to the PFGE inbox (PFGE@cdc.gov). Once all isolates have been submitted either to the online

database or to the PFGE inbox, it is only necessary to provide one isolate with the outbreak pattern on the WebBoard. Remember, "matches" must be verified by CDC PulseNet before they can be counted as part of the outbreak.

Q. When performing online queries, why does the connection sometimes time out?

A. The main reason the connection 'times out' is the computer is performing calculations on a large number of patterns, or is trying to download many patterns at once. The key is to limit your queries to only those patterns you really want to view. For example, when querying the *Salmonella* database, first query for the serotype of choice and then query within that serotype. To query within a 60-day 'hot list' you would first 'Select Hot List' and before downloading the entries, you would then 'Search in List.' Also remember to 'Delete Current Selection' or 'Replace List' when you want to perform a new query. These scenarios will give you a smaller number of patterns with which to work, and will hopefully prevent the 'time out.'

Q. How can I find out which patterns have been associated with outbreaks, past and present?

A. This can be done through querying the 'Outbreak' field on the on-line server. Any outbreaks or clusters from fall of 2002 until now will have the new outbreak code. Any outbreaks before fall of 2002 will have the common name for that outbreak. For more recent outbreaks and clusters, if you are unsure of the code, but would like to see all the isolates involved in an outbreak in, for example, 2003, you could use the query '03*' in the 'Outbreak' field. This will bring up all outbreak or cluster associated

patterns for 2003. All outbreaks and clusters since PulseNet's inception are not coded. However, we are striving to provide that code for current outbreaks and clusters as well as working on filling in the field for those of the past. **CDC**



TECHNICAL TIPS FOR PERFORMING PFGE

- Use gentle pipetting during plug preparation. For *Campylobacter* PFGE, prepare plugs ASAP after removing plates from the incubator—*Campylobacter* is very sensitive to its environment.
- If your laboratory only has frost-free freezers (a 'frosty' or non-frost freezer is preferred), place enzymes in an insulated storage box (these are often referred to as laptop or benchtop coolers) in the freezer center.
- When capturing gel images make sure to fill the entire viewing field with your gel.
- The PulseNet Task Force at CDC recommends PFGE training for at least one additional lab person to assist during outbreaks.
- Changing the source of your laboratory's reagent grade water and/or TBE (commercially prepared vs. lab-prepared from individual ingredients) may affect electrophoresis currents and optimum run times.
- Test new lots of *Salmonella* Braenderup H9812 standard PFGE plugs by restricting and running a representative sample of the new plugs with an "old" plug to be sure that the PFGE pattern and band intensities are the same. Use the *Salmonella* electrophoresis conditions to test the H9812 plugs.

HOW WOULD YOU LIKE TO RECEIVE THE PULSENET NEWSLETTER?

The newsletter is made available electronically on the PulseNet website at: (www.cdc.gov/pulsenet/news.htm). If you would like to stop receiving the hard copy version and either receive the electronic version via e-mail or access it via the website, please send your request to the PFGE inbox at pfge@cdc.gov with the subject line: PulseNet Newsletter.

(Continued from page 2) the reality is that clinical microbiology is a highly experience-dependant field where so-called expert instrumentation requires the oversight of well-trained clinical scientists.

Our response to this reality has been to institute an extensive outreach program to our clinical microbiology laboratories. The goal is to have every clinical microbiologist in the state be on a first name basis with at least one microbiologist at the BOL. We have 3.5 Full Time Employees (FTEs) involved in outreach who provide continuing education through web-based and CD-ROM courses and centralized and regional wet-workshops. The FTEs also travel to individual laboratories as needed, to address susceptibility testing, biosafety, parasitology, identification of agents of bioterrorism, mycology, packaging and shipping regulation compliance, and foodborne outbreak investigation training (including collection of specimens for testing) for sanitarians and communicable disease nurses at local health departments. Workshops are often co-sponsored by the National Laboratory Training Network.

This continuing education for clinical microbiologists has been further enhanced by our laboratory newsletter, the *LabLink*, and by a broadcast fax system which we developed from a database of all clinical microbiology laboratories in the state. New testing developments or information of an emergent nature, such as the recent outbreaks involving SARS and the *E. coli* O157:H7 associated with bacon-wrapped steaks, are communicated real-time through the fax system. This real-time communication provides not only an important source of information to the clinical laboratories and their hospitals, but helps the clinical microbiologists identify their value within the public health system. It has proven to be a valuable tool, providing a sense of inclusion for clinical microbiologists and acknowledging the essential nature of their contribution to our work and the health of the community. These interactions cover many topics. On the surface, our interaction with clinical microbiologists appears to have little association with our PulseNet activities, but any personal contact enhances the knowledge of clinical microbiologists about the activities of our public health laboratories and improves communications, which is, after all, what this is all about. **CDC**

Utah

View of Olympic village at the base of the Rocky Mountains from the Utah Department of Health's laboratory

Utah Department of Health's (UDOH) participation in PulseNet began in 1996 when Dr. Susan Mottice attended the first PFGE training course at CDC. By 1998 Chef Mappers had been purchased, reagents and supplies stocked, and Utah was designated as a PulseNet area lab. Dr. Mottice, who was Utah's Microbiology Bureau Director and Darren Pearce, Molecular Biology Section Chief, represented Utah at the 1998 PulseNet Update meeting in Seattle, Washington. They obtained

useful information about PFGE and were excited about being a part of such an important group. Now, five years later, Utah serves as an area lab for Arizona, Colorado, Montana and Wyoming and remains enthusiastic about PFGE and its future. Currently, Jenni Wagner serves as the PFGE Microbiologist; Barbara Jepson is the Microbiology Bureau Director, and Dr. June Pounder is Chief of the Molecular Biology Laboratory.

Throughout Utah's history with PFGE, we have been involved with many interesting cases, which were found during routine subtyping and foodborne outbreak surveillance of *Salmonella*, *Listeria*, *E. coli* O157:H7, *Shigella* and *Campylobacter* isolates. In 2000, an unexpected number of infant botulism submissions were seen in the lab, which raised concern about their source. When PFGE results of the *Clostridium botulinum* isolates clearly showed no relationship between the samples, hysteria about contamination of a commercial food source was alleviated. Jana Coombs began PFGE subtyping *Campylobacter jejuni* isolates in

Utah prior to the establishment of the CDC national database. When the lab tested *Campylobacter* samples from Northern Utah high school football

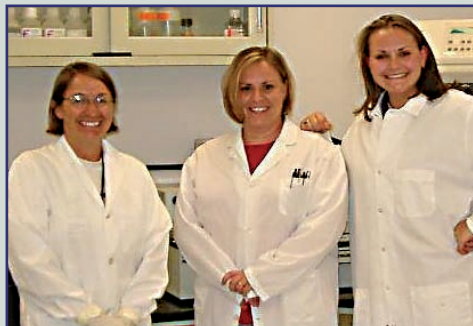
players and found that they were related, the source was determined to be contaminated non-culinary irrigation water that the football players were drinking during training camp.

Scott Weidner was trained in PFGE at CDC in the fall of 2001; he held the PFGE Microbiologist position for one year at the UDOH and was certified for *E. coli* T1FFs and analysis. When Scott returned to school, Jenni Wagner then came to CDC for PulseNet PFGE training. Recently, she has been working with local physicians and epidemiologists on a project to subtype Oxacillin-resistant *Staphylococcus aureus* (ORSA) because of concerns about recent outbreaks of ORSA in homeless and incarcerated populations in neighboring states. A project to test the homeless population client base and determine relatedness of ORSA isolates and possible transmission patterns at Utah's Fourth Street Clinic, which serves the indigent in Salt Lake City, has begun.

The UDOH PFGE facility is housed in the Molecular Biology lab. The PFGE Microbiologist also serves as a Bioterrorism Response Team member and supports all testing in the Molecular Biology lab. In-house PFGE protocols for *Bordetella pertussis*, *Haemophilus influenzae*, and *Streptococcus pyogenes* have been developed. In 2000, Jana Coombs presented a report on PFGE subtyping of Invasive *Haemophilus influenzae* A at the PulseNet Update Meeting. UDOH plans to implement PFGE of bioterrorism agents when protocols become available. Jenni has installed

BioNumerics software and converted all *E. coli*, *Listeria*, *Salmonella*, and *Campylobacter* Molecular Analyst data to this format.

As an area lab, Utah is trying to keep up with the changing needs of Utah and its neighboring states. PulseNet's 2003 Update Meeting provided great opportunities for discussion and suggestions about the area lab's role. We have begun regular conference calls to improve our communication and update all labs in Utah's area, with the first one held on July 28, 2003. These calls will help determine concerns of each lab within the area and how we can help each other. The update meeting in San Antonio showed how much can be accomplished with cooperation. In order to provide real-time testing for *Listeria* samples, all labs within the area can send their isolates for testing to the UDOH. We hope to provide training, additional testing, surge capacity, and leadership for the labs in our area.



(From left to right) June Pounder, Kim Christensen and Jenni Wagner make up the Molecular Biology laboratory

This background image is a view of downtown Salt Lake City and the Great Salt Lake from the laboratory

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Publications and Abstracts

- Graves L, Hunter S, Hise K, Kornstein L, Schoonmaker-Bopp D, Head M, Jones J, Papedis K, Ahanotu E, Gottlieb S, and Swaminathan B. **Laboratory Investigation of a Multistate Outbreak of Listeriosis in the Northeastern United States**, 2002. 90th Annual Meeting of the International Association for Food Protection (IAFP), presented in New Orleans, Louisiana, August 10-13, 2003.
- MMWR Morb Mortal Wkly Rep. 2003** July 4;52(26):613-615. Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurized milk—Illinois, Indiana, Ohio, and Tennessee, 2002-2003.

CDC PulseNet Task Force New Members

PulseNet National Database Administration Team:

- Desmond Jennings** joined the PulseNet database administration team in early September 2003. He graduated from the University of Georgia in 2002 with a B.S. in Biology. Since graduation, Desmond has been a veterinary

assistant at a local veterinary hospital. Desmond will be the new *Shigella* database manager.

PulseNet Methods Development/ Validation Laboratory:

- Paola Bordoni** joined the PulseNet team in September 2003. She graduated in 2002 from Boston University with a B.S. in Biology/Psychology. Paola will be working in the *Listeria* laboratory and will also be working part-time on the *Listeria* national database.
- Kristan Kiser** joined the PulseNet team in early August 2003. She graduated from the University of Georgia in 2001 with a B.S. in Microbiology. Before coming to CDC, Kristan worked in the Virology Quality Control Lab at Merial in Athens, GA.
- Melissa Butler** joined the PulseNet team in August 2003. She graduated from the University of Georgia in May 2002, with a B.S. in Biology. After graduation, she was a member of a lab in the Department of Microbiology at UGA which researches the symbiotic rela-

tionship of a luminescent marine bacterium, *Vibrio fischeri*, and the Hawaiian bobtail squid, *E. scolopes*.

CDC PulseNet Task Force Farewells

- Dr. Peter Gerner-Smidt**, a visiting scientist from the Statens Serum Institut in Copenhagen, Denmark, returned home July 31st after a year of working with the PulseNet Database Administration Team. Peter was at CDC to facilitate the creation of PulseNet Europe and

was supported by a WHO fellowship. He assisted with the *Listeria* outbreak last summer (2002) and worked with the *E. coli* database. He participated in the 7th Annual PulseNet Update Meeting in San Antonio and gave an informative presentation on pattern interpretation. Peter's knowledge and wisdom have been great assets to the PulseNet Database Administration Team and he will be sorely missed.

- Kim Hutcheson Lockhart**, a member of the PulseNet Methods Development and Validation Laboratory since September 2002, left CDC in July 2003 to teach 7th and 9th grade science at a private school. She is also coaching boy's soccer and will help establish the girl's soccer program. In addition to doing routine PFGE of *E. coli* and *Shigella* isolates, Kim was responsible for setting up the validation study of the PulseNet Standardized PFGE protocols for *Vibrio cholerae*, *V. parahaemolyticus*, and *Yersinia enterocolitica* and lyophilizing and testing *Campylobacter* strains, which will be used for PulseNet Certification. We wish Kim all the best in her teaching career.

STATE, COUNTY AND CITY
HEALTH DEPARTMENTS

State, County and City Public Health Laboratories

From around the nation, we welcome:

- Amanda Moore**, who recently joined the Molecular Epidemiology Laboratory Section at the South Carolina Department of Health and Environmental Control. She is a Laboratory Technologist II and will be learning PFGE.
- Jason Herstein**, who joined the Microbiology Department of the Missouri State Public Health Laboratory as an Associate Public Health Scientist.