2005 PulseNet Update Meeting
Poster Listing and Abstracts

1. **Use of Pulsed- Field Gel Electrophoresis (PFGE) for *Bordetella pertussis* Related Surveillance and Outbreak Investigation. T. A. Kurzynski[1]**, D. M. Warshauer[1], P. K. Cassiday[2], J. P. Davis[3] and T. A. Monson[1]. [1]Wisconsin State Laboratory of Hygiene, Madison, WI, [2]CDC, Atlanta, GA and [3]Wisconsin Department of Health and Family Services, Madison, WI.

   Background: The high incidence of *Bordetella pertussis* (Bp) infections in Wisconsin (WI) in 03-04 provided an opportunity to examine the use of pulsed-field gel electrophoresis (PFGE) for surveillance and outbreak investigations. **Conclusions:** PFGE subtyping was a useful discriminatory tool to establish distribution patterns of individual strains circulating in a limited geographic region during a large community wide outbreak. The WI 03-04 isolate PFGE profile distribution was different from the PFGE profile distributions among WI archival isolates and the recent national (CDC) isolates. Thus, different profiles can predominate during different time periods in a particular region and a local *B. pertussis* population structure may not reflect national trends, especially under outbreak conditions.

2. **Pulsed-Field Gel Electrophoresis Analysis of *Campylobacter jejuni* Isolates in South Dakota. Christopher Carlson** and Nick Hill.

   Abstract: The most common enteric bacterial disease reported to the Department of Health in 2004 was Campylobacteriosis. The number of *Campylobacter* cases was 69% over the 5 year average and nearly double all of the other common food borne infections (*E. coli* O157:H7, *Salmonella, Shigella* and *Listeria*) combined. Although routine subtyping of Campylobacter is generally not recommended, it was decided to look retrospectively at stored isolates to determine if PFGE patterns could have been used as part of the epidemiological investigations. PFGE analysis was carried out using the standardized PulseNet procedure. The primary enzyme used for restriction was SmaI. A total of 128 patterns were obtained with 63(49%) of those belonging to 13 clusters with 3 or more indistinguishable patterns per cluster. A majority of the larger clusters occurred during or around the month of June. Cases occurred statewide however a larger portion of cases were found in the eastern part of the state which also has larger population size. Epidemiologic case investigations did not implicate any particular exposure or identify any point source outbreaks. Interview information was obtained from 96(75%) of the cases with PFGE data. A couple of risk factors stood out with 42% of cases reported exposure to poultry, raw or undercooked, and 72% reported exposure to animals. Animal exposure included household pets and/or livestock. With 49% of the PFGE patterns belonging to certain clusters, this information may have been helpful to investigators when conducting the interviews. Those cases belonging to a cluster would have been given a more detailed questionnaire to try and obtain important information.

3. **The Role of Pulsed-Field Gel Electrophoresis in Investigating a *Salmonella* Outbreak Linked to Roma Tomatoes.** Carol H. Sandt, Donna A. Krouse, Charles R. Cook, Amy L. Hackman, and **Wayne A. Chmielecki**

   Abstract: In July 2004 the PA Dept of Health (PA DOH) and the PA Dept of Agriculture (PDA), along with the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA), were involved in the investigation of a multi-state foodborne outbreak of *Salmonella*. The outbreak, centered in Pennsylvania, involved food items purchased at deli counters of a large gas station chain. Routine surveillance by the PA DOH's pulsed-field gel electrophoresis (PFGE) lab showed a spike in serotype Javiana, and PFGE analysis of these isolates identified an outbreak

pattern. An epidemiologic investigation implicated tomatoes as the source of infection. From tomatoes the PDA isolated *Salmonella*, which the PA DOH rapidly serotyped as Anatum. Subsequently, PFGE established a direct patient-tomato link for this organism. As the epi investigation and PFGE testing continued, four serotypes (Javiana, Anatum, Thompson, and Typhimurium) were eventually implicated. PFGE results failed to support the involvement of one epi-linked serotype (Muenchen). The standardized CDC PFGE protocol (Ref. 1) using multiple enzymes was used to further characterize the isolates. Through the CDC-sponsored PulseNet communications system, PFGE results were posted to the WebBoard. Rapid responses from neighboring states revealed this to be a multi-state outbreak.

4. **PFGE analysis of *Campylobacter* sp. isolated from humans and retail foods of animal origin. David C. Melka**, David G. White, Linda English, Sherry Ayers, and Shaohua Zhao. Food and Drug Administration – Center for Veterinary Medicine, Office of Research, Division of Animal and Food Microbiology, 8401 Muirkirk Rd. Laurel, MD 20708.

   Background: *C. jejuni* and *C. coli* are closely related bacterial species that are associated with a large number of human gastroenteritis cases worldwide. Most campylobacter infections are sporadic single cases resulting from the consumption of contaminated food, milk or water. Therefore, it is important to understand their molecular epidemiology and evolution.

5. **Characterization of *Salmonella* Typhimurium of Animal Origin Obtained from the National Antimicrobial Resistance Monitoring System (NARMS). S. Zhao[1*]**, P.J. Fedorka-Cray[2], S. Friedman[1], P.F. McDermott[1], R. D. Walker[1], S. Qaiyumi[1], S.L. Foley[1**], S.K. Hubert[1], S. Ayers[1], L. English[1], D.A. Dargatz[3], B. Salamone[4] and D.G. White.[1] [1]Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U. S. Food & Drug Administration, Laurel, MD; [2]USDA, Antimicrobial Resistance Research Unit, Agricultural Research Services, Athens, GA; [3]USDA, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, Fort Collins, CO; [4]USDA, Food Safety and Inspection Service, Zoonotic Diseases and Residue Surveillance Division, Washington, DC

   Background: *Salmonella* Typhimurium remains one of the most common causes of salmonellosis in animals and humans in the United States. The emergence of multi-drug resistant *Salmonella* reduces the therapeutic options in cases of invasive infections, and has been shown to be associated with an increased burden of illness. **Conclusions**: Results demonstrated a varied spectrum of antimicrobial resistance, including several multidrug resistant clonal groups, among *S.* Typhimurium and *S.* Typhimurium var. Copenhagen isolates recovered from both diagnostic and slaughter/processing samples.

6. **Comparison of Multi-Locus Sequence Typing, Pulsed-Field Gel Electrophoresis, and Antimicrobial Susceptibility Profiles for Characterization of *Salmonella enterica* Serotype Newport Isolates from Human and Food Animal Infections and Retail Meat**. H. Harbottle, **S. Zhao,** D. G. White, P. F. McDermott, and R. D. Walker. Center for Veterinary Medicine, U. S. Food and Drug Administration.

   Abstract: Several *Salmonella enterica* serotypes are a major source of food borne illness in the United States accounting for over 1.4 million cases of human illness yearly. Multi-drug resistant phenotypes of serotype Newport (MDR-AmpC) have emerged in animals and humans and have become a major public health problem. Pulsed-field gel electrophoresis (PFGE) and antimicrobial susceptibility profiling methods are commonly used methods for studying microbial epidemiology and trends in antibiotic resistance of bacteria. In this study, 82 *S.* serotype Newport isolates were analyzed by PFGE, antimicrobial susceptibility profiles, and multi-locus sequence typing (MLST) of seven

genes, *aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*. PFGE profiling resulted in 44 fingerprint types, and five of these fingerprints were indistinguishable among animal and human isolates. Antimicrobial susceptibility testing results show that 37.8% of the 82 S. serotype Newport isolates were considered to be MDR-AmpC and 21 resistance profiles were identified. MLST resulted in 13 sequence types, 11 of which were novel, with one sequence type encompassing 57.3% of the strains. Five novel allele types were identified. These results demonstrate that the MLST scheme employed here did not add any discriminatory power to the profiling of these isolates, and discriminated poorly between isolates that were separated well by PFGE. In this study PFGE and antimicrobial susceptibility profiling were the most useful tools for discriminating between isolates and tracking *Salmonella enterica* serotype Newport infections in humans and animals.

7. **The PulseNet *E. coli* O157:H7 National Database Year in Review, 2004**. **M.M. Joyner**, J.A. Kincaid.

   Description: This poster presents a summary of activity within the *E. coli* national database in 2004. It contains information regarding the number of laboratories that were certified for analysis of *E. coli* and that submitted data during 2004, gives an overview of the number of patterns submitted to the *E. coli* national database in 2004, and provides the top 5 patterns seen in the database during 2004. It also summarizes source type information, the breakdown of clusters detected monthly, and 2004 outbreak highlights. In addition, it presents objectives for the *E. coli* national database for 2005.

8. **The Significance of Second Enzyme Data, PulseNet National *E. coli* O157:H7 Database**. M.M. Joyner, **J.A. Kincaid**.

   Description: This poster presents PulseNet's recommendation for the use of second enzyme when performing PFGE on isolates of *E. coli.* It also displays the results of a survey issued to PulseNet laboratories regarding the use of second enzyme with *E. coli.*

9. **PulseNet Quality Assurance and Quality Control (QA/QC) Program: An Update**. **J. Kincaid,** C. Steward, and D. Sheehan.

   Description: An overview of PulseNet's QA/QC program, including purpose, current status, tips and reminders, and other useful certification and proficiency testing information.

10. **Comparison of a Multiplex PCR Assay and Conventional Serotyping for Sero-classification of *Listeria monocytogenes* Isolates**. **Lewis M. Graves**, Wallis DeWitt, Lynn Mauro, Paola Bordoni, and Bala Swaminathan.

    Abstract: A recently described multiplex PCR assay (Doumith, et al, J. Clin. Microbiol. 42: 3819-3822, 2004) was evaluated against conventional serotyping using a set of 80 *Listeria monocytogenes* isolates. The multiplex PCR assay clusters *L. monocytogenes* isolates into four major groups (group 1: serotypes 1/2a, 3a; group 2: 1/2b, 3b, 7; group 3: 1/2c, 3c; group 4: 4b, 4d, 4e). *Listeria* isolates that do not group into one of the four major serotypes are designated "L". The eighty *L. monocytogenes* isolates used for this study included forty-eight clinical *L. monocytogenes* surveillance isolates that were received in the *Listeria* Reference laboratory between January 6, 2005 and February 1, 2005 and 32 retrospective isolates collected between January 1, 2000 and December 29, 2004. The retrospective isolates were untypable by conventional serotyping. The concordance between the two methods for the surveillance isolates was 94%. For the retrospective isolates untypable by conventional serotyping, the multiplex PCR method was able to classify 88% of the isolates into one of the four major groups. The multiplex

PCR assay is useful for rapid routine screening of *L. monocytogenes* in PulseNet participating laboratories.

11. **PulseNet's National *Listeria* Database: 2004 Year in Review**. **P. Bordoni**, L. Graves, L. Mauro.
    Description: This poster will give a summary of the *Listeria* database as of December 2004 and a description of *Listeria* Outbreaks in 2004.

12. **PulseNet *Salmonella* National Database Year in Review, 2004. JL Lockett**, BM McGlinchey, MS Van Duyne, NJ Patel.
    Description: This poster will visually describe the National *Salmonella* database for the year of 2004. The information includes statistics on submitting laboratories, distribution of samples, top five serotypes and notable outbreaks of 2004. "*Salmonella* Year in Review" poster also states goals for 2005.

13. **Multistate Outbreak of Salmonellosis Associated with Roma tomatoes—Mid-Atlantic, 2004**. **NJ Patel**, MS Van Duyne, JL Lockett, BM McGlinchey.
    Abstract: There are approximately 1.4 million *Salmonella* infections annually in the US. PulseNet USA detects multi-state and regional clusters and outbreaks by the use of pulse-field gel electrophoresis (PFGE) of foodborne pathogens, and assists in the investigation of potential common source outbreaks. On July 14, 2004, PA Department of Health noted a cluster of *Salmonella* Javiana cases in PA involving food from Gas Station Deli Chain A, and notified the PulseNet USA Administration Team. With the use of the PulseNet WebBoard communication tool, PFGE results were posted and rapid responses from nation-wide public health laboratories revealed this to be a multi-state, multi-serotype outbreak. An epidemiological investigation indicated Roma tomatoes from the Gas Station Deli Chain A as the common source of the outbreak. Additional epidemiological investigations and PFGE results confirmed three additional serotypes: *Salmonella* Anatum, Typhimurium, and Thompson. A number of isolates collected with indistinguishable PFGE patterns continued to be collected until August 2004. Illness onset after the incubation period of *Salmonella* could be due to factors such as continued Roma tomato use, poor recall, low infectious dose, secondary transmission, or food saved and eaten later.

14. **PulseNet *Shigella* National Database 2004 Year in Review D. Jennings**
    Description: This poster will give a summary of the *Shigella* database as of December 2004 and a brief description of the *Shigella sonnei* outbreak associated with air travel from Hawaii that occurred in 2004.

15. *CDC board*

16. ***Campylobacter jejuni* Year in Review, PulseNet National Database, 2004**. **Merritt Adams**, Ciara O'Reily, Steve York, Wanda Manley, Jenni Wagner.
    Description: This poster will be a review of the stats, notable outbreaks, and common patterns for the *Campylobacter* National Database.

17. *CDC board*

18. **Are cases of campylobacteriosis less sporadic than they might appear? – Application of PFGE, Penner serotyping, GIS mapping, and epidemiological investigation to understanding campylobacteriosis in New Zealand. Brent Gilpin**, **Beth Robson**, Angela Cornelius, Alan Ferguson, Tom Henderson*, Naomi Boxall, and Carolyn Nicol. Institute of

Environmental Science & Research Limited, New Zealand. *Medlab South Ltd, Christchurch New Zealand. Contact: Brent.Gilpin@esr.cri.nz.

Abstract: Campylobacteriosis is a notified disease in New Zealand, with a reported rate of in 2004 of 327.4 cases per 100,000. Despite being the most common bacterial disease in New Zealand, and many other countries, outbreaks of campylobacteriosis are rarely identified. One reason for this is that subtyping methodology is infrequently applied to isolates. To evaluate the potential application of laboratory subtyping of *Campylobacter*, 177 isolates of *Campylobacter* were collected from a single clinical laboratory - 78 during an eight week period in Spring, and the rest two months later over a second eight week period in Autumn. Isolates were subtyped using Penner serotyping (*C. jejuni* only) and PFGE using both *Sma* I and *Kpn* I. PFGE patterns of isolates were compared with isolates from a range of human, animal and environmental sources that had been submitted to the PulseNet Aotearoa New Zealand *Campylobacter* database (>1200 isolates, March 2005). Clusters of between 2 and 26 isolates with identical *Sma* I, *Kpn* I, and Penner serotypes were identified. To evaluate the validity of the clusters 168 of the isolates could be linked retrospectively to notified epidemiological data collected as part of the New Zealand notifiable disease database, and all isolates were GIS mapped on the basis of at least their home address. Although the epidemiological data was incomplete, potential linkages could be made between many of the cases clustered by laboratory typing of isolates. This pilot study suggests that the combined, and timely application of PFGE, epidemiological questionnaires and GIS mapping of cases of campylobacteriosis may be a useful approach to unraveling the sources responsible for many of the cases of campylobacteriosis in New Zealand.

19. **Use of Pulsed-Field Gel Electrophoresis (PFGE) to confirm Foodborne Outbreaks through PulseNet partnership: Alberta's 5-year Perspective. L. Chui[1]**, M. Louie[1], G. Tyrrell[1], L. Honish[2], I. Zazulak[2], L. Crowe[3], Outbreak Investigation Committee and Alberta Health and Wellness. [1]Provincial Laboratory for Public Health (Microbiology), [2]Capital Health-Public Health Division, [3]Calgary Health Region.

20. **Pheno- and genotypic characterisation of *Salmonella enterica* Paratyphi B isolates from environmental and human sources in Galicia (north-west Spain). Jaime Martinez-Urtaza**[1], Aurora Echeita[2], and Ernesto Liébana.[3] *Instituto de Acuicultura, Universidad de Santiago de Compostela, , Spain [1]; Laboratorio Nacional de Referencia de Salmonella y Shigella, Instituto de Salud Carlos III, Spain[2]; and Department of Food and Environmental Safety, Veterinary Laboratories Agency-Weybridge, United Kingdom.[3]*

Abstract: *S. enterica* serotype Paratyphi B isolates from gastrointestinal infections (enteric variant) are normally d-tartrate fermenters (dT^+), while those obtained from cases of human enteric fever (systemic variant) normally fail to utilize this substrate (dT^-). Information relative to the natural reservoirs of *S.* Paratyphi B is scarce, particularly in reference to the survival and transmission routes of the dT^- variant. In this study, we have characterized *S.* Paratyphi B isolates originating from the marine environment and human infections in Galicia (NW Spain). We subsequently have analysed the degree of genetic diversity and clonal relationships among isolates. Four shellfish isolates, and all (n=8) human isolates from this region were included in the study. In addition, four reference strains from Germany and the UK were incorporated for comparison. D-tartrate fermentation was investigated by both lead acetate test and PCR. The presence of genes encoding the effector proteins sopE1 and avrA was tested by PCR. *Xba*I-pulse-field gel electrophoresis and antimicrobial susceptibility testing to 16 drugs were also carried out. Three isolates obtained from mussels and oysters between 1998 and 2001 were dT^-, while a single isolate obtained from mussels in 2002 was dT^+. All four isolates originated from the same area (ria of Arousa). Two human isolates from blood and stool samples,

isolated during 2000 in the city of Ourense were dT^-. The remaining 6 human isolates were dT^+. All dT^- isolates were *sopE1*positive and *avrA* negative. By contrast, dT^+ isolates were all *sopE1*-negative and showed heterogeneous results when the *avrA* gene was investigated. All the dT^- isolates presented an indistinguisable PFGE profile. This cluster included all the Spanish isolates as well as the dT^- reference strains from Germany and UK. This suggests that all these isolates could belong to the globally distributed clone Pb1. Limited PFGE heterogeneity was also observed among dT^+ isolates from Galicia. The PFGE profiles of these isolates were clearly different from those from reference isolates from Germany and the UK. Three human dT^+ isolates showed an indistinguishable PFGE profile to the dT^+ mussel isolate. Antimicrobial susceptibility testing showed that 13 strains were sensitive to all 16 antibiotics in our panel, while three isolates were resistant to a single antibiotic. The identification of *S.* Paratyphi B isolates of the systemic variant associated with shellfish has revealed a significant survival capacity of this organism in high salinity environments. This finding is of notable public health significance, since it indicates the potential risk of acquiring enteric fever linked to the consumption of raw shellfish. Furthermore, the presence of indistinguishable PFGE fingerprints in dT^+ isolates from human and shellfish origin, indicates also the risk of zoonotic infection by the enteric variant of this serovar.

21. Use of Pulse-Field Gel Electrophoresis to discriminate among the dominant clinical clones of Vibrio parahaemolyticus. J. Martinez-Urtaza, L. Simental, A. Lozano-Leon *Instituto de Acuicultura, Universidad de Santiago de Compostela, Santiago de Compostela, SPAIN*.

Abstract: *Vibrio parahaemolyticus* is a halophilic member of the genus *Vibrio* that inhabits temperate and tropical marine environments worldwide. Strains producing the TDH and/or the TRH hemolysins are considered pathogenic. Recently, *V. parahaemolyticus* infections have increased globally. The new O3:K6 clone is considered the first *V. parahaemolyticus* pandemic in history. This clone emerged in 1996 and from this date, it has accounted for the majority of *V. parahaemolyticus* infections in Asia, spread to the USA and Chile. The pandemic clone also includes isolates belonging to serotypes O4:K68, O1:KUT, O1:K25 and O1:K41. Recently, serotype O4:K11isolates obtained from human infections in Spain and UK were identified as belonging to a unique and specific clone. In this study we evaluated the use of PFGE to identify the clonal relationship of three clinical isolates obtained from a recent foodborne outbreak in A Coruña (NW Spain). The three outbreak isolates, Spanish clinical and environmental isolates, isolates belonging to the different serotypes included in the pandemic clone, and O3:K6 non-pandemic strains were included in this study. All the isolates were subjected to PFGE analysis by NotI. PFGE analysis grouped the different isolates according to their reported characteristics. All the isolates belonging to the pandemic strains showed very close restriction patterns with slight differences and were grouped in a cluster with a similarity percentage >95%. The four O3:K6 non-pandemic strains isolated in different Asian countries before 1995 were clearly discriminated from the pandemic clone isolates and grouped in a same cluster with a similarity percentage >80%. European clinical isolates from previous outbreaks were discriminated in two closely related PFGE-types included in a homogeneous cluster, clearly differentiated from the pandemic and non-pandemic isolates. The Spanish environmental isolates and the ATCC reference strains showed distinct and unrelated restriction patterns. The three isolates obtained from the recent outbreak of illnesses in Galicia presented identical PFGE patterns, indistinguishable from the pandemic O3:K6 strains isolated from India, Laos, Bangladesh, Taiwan and Thailand between 1996 and 1998, and closely related to the other pandemic strains included in the study, and they were unequivocally identified as belonging to the pandemic clone. These results were subsequently confirmed by

additional serotyping and molecular analysis. PFGE has shown to be a reliable method to distinguish among the main clinical clones of *V. parahaemolyticus* and a useful tool for a rapid clone-related identification of isolates from foodborne outbreaks and human infections. Data from PFGE analysis and epidemiological information provide us the first evidence that the pandemic *V. parahaemolyticus* clone has been introduced in Europe.

22. **Characterisation of blaCTX-M genes in clinical isolates of Salmonella recovered from humans during the period 1992-2003 in England and Wales**. **E. Liebana[1]**, **M. Batchelor[1]**, K. Hopkins[2], E. J. Threlfall[2], F. A. Clifton-Hadley[1], A. Stallwood[1], R. H. Davies[1]; [1]Veterinary Laboratories Agency, Addlestone, Surrey, UNITED KINGDOM, [2]Health Protection Agency, Colindale, London, UNITED KINGDOM.

23. **Present State of Development of PulseNet Asia Pacific.** Yiu Wai CHU, Cindy KY LUEY, Danny TL CHEUNG, Man Yu CHU, Agatha WH CHIU, Susanna FS LEUNG, Choi Ha MA & Kai Man KAM on behalf of all the participants of PulseNet Asia Pacific.

Abstract: PulseNet Asia Pacific has made much progress, since the first Planning Meeting in Honolulu in December 2002. We have now drawn together thirteen countries and areas within the region with a population totaling at 2.9 billion as estimated in 2001. Against a backdrop of enormous cultural and social economic differences, we are currently working hard in bringing closer the participants in terms of standardizing methods and communication protocols. A survey among the participants has identified a priority list of pathogens for our region with *Salmonella* (including *S.* Typhi and non-typhoidal salmonellae) ranked number one. In order to provide impetus to tackle some of these issues, four work groups, namely, Laboratory Resources and Support, Salmonella Subtyping, Server Development and Platform for Interlaboratory Comparison have been created and coordinated by Japan, Australia, New Zealand and Hong Kong respectively. In view of diversity of background and differing pace of laboratory development, training for the CDC standardized one-day protocol has also been recognized as very important. The second PulseNet Asia Pacific Training Workshop was held in March 2005 to meet such a need. Scientists from China, South Korea, Thailand and Vietnam converged in Public Health Laboratory Centre in Hong Kong for a four-day hands-on workshop conducted by trainers from CDC, USA, the National Institute of Infectious Diseases (NIID), Japan and Department of Health, Hong Kong.

24. **An Overview of PulseNet Canada achievements. Lai-King Ng** and PulseNet Canada members. Bacteriology and Enteric Diseases Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada, BC Centre for Disease Control, ProvLab Alberta, Provincial Laboratory Saskatchewan Health, Cadham Provincial Laboratory (Manitoba), Ontario Central Public Health Laboratory, Laboratoire de Santé Publique du Québec, Saint John Regional Hospital (New Brunswick), Queen Elizabeth II Health Sciences Centre (Nova Scotia), Provincial Health Laboratory Queen Elizabeth Hospital (Prince Edward Island), Newfoundland Public Health Laboratory, Laboratory for Foodborne Zoonoses (PHAC), and the Bureau of Microbial Hazards Food Directorate (Health Canada).

Abstract: Federal and provincial laboratories that perform Pulsed Field Gel Electrophoresis for PulseNet Canada databases adopted the standardized protocols of the CDC. Gel images are normalized using scripts written by Applied Maths (Belgium) for BioNumerics, and unique patterns are submitted to the national database at the National Microbiology Laboratory for designation. Clusters of strains of indistinguishable PFGE patterns or outbreaks under investigation are communicated to laboratory network members, epidemiologists and the CDC. Recently, the National Microbiology Laboratory replaced the listserv with an in-house designed web-tool to facilitate

exchange of information. Standardization of methods, and rapid sharing of information through the internet has reduced the time and effort spent identifying outbreaks. The activities of PulseNet Canada help differentiate outbreak strains from sporadic strains. This data is also used in epidemiological investigations to determine the cause of outbreaks, and has subsequently increased the power and sensitivity of our surveillance system. In the past few years, there are many examples that illustrate how the establishment of PulseNet has reduced the impact of outbreaks. Some investigations have resulted in the recall of contaminated food, change of food handling or processing practices, environmental interventions, regulation and policy changes or public health interventions. In addition to improved laboratory methods and availability of internet communications, collaborative efforts from teams of professionals in different jurisdictions, disciplines and international organizations have played significant roles in reducing the risk of food-borne and water-borne pathogens.

25. PulseNet Europe. **Susanna Lukinmaa**, Statens Serum Institute, Copenhagen, Denmark.

Abstract: PulseNet Europe is a network for the molecular surveillance of food-borne infections in Europe. It is an internationally unique network that includes besides of public health laboratories also laboratories from the veterinary and food sector as equal partners. This equal participation and collaboration will strengthen the surveillance and make the combat against food-borne infections more efficient. The current objectives in PulseNet Europe is to establish real-time linked surveillance database system to detect infection clusters and investigate outbreaks of *Salmonella*, verocytotoxigenic *E. coli* (VTEC) and *Listeria monocytogenes,* set up a central database, train database curators, prepare Memorandum of Understanding with PulseNet International and establish a rapid communication system. Setting up PulseNet Europe has been facilitated by the experiences from a number of other networks, such as PulseNet USA (http://www.cdc.gov/pulsenet/), the European public health network EnterNet (http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm), and the Salm-gene project (http://www.salmgene.net). At the moment, PulseNet Europe receives funding to establish the core infrastructure with the current objectives, as a work package in the FP6 network of excellence MedVetNet, a virtual European Zoonosis Centre (http://www.medvetnet.org/). However, the funds are not aimed at training partners, setting up certification system, sustaining and developing database. Therefore, such funds are applied from EU and the current application includes participants from 54 Institutions from 28 European countries. The Steering committee for PulseNet Europe was established in September 2004. It has eight members: the project coordinator, the six PulseNet Europe database curators and the expert in bioinformatics who is in charge of building and maintaining the database system. The typing method presently used is pulsed-field gel electrophoresis (PFGE). However, one of the future issues for PulseNet Europe is the integration of new alternative subtyping methods to be used in the network. The structure of the PulseNet Europe database is built in the BioNumerics server/client format and the on-line database server is at the Health Protection Agency, Colindale, England. The curators are connected to this central database. The system will be set up so that profiles will be submitted both to the on-line server and to the designated curator. The curators will be responsible for analysis of profiles submitted from the participants, naming and confirming the profiles, and for uploading the confirmed profiles to the on-line central database. The data in PulseNet Europe database will be also comparable internationally through PulseNet International making the molecular surveillance global.

26. *PulseNet Latin America board*

27. Identification of Single-Nucleotide Polymorphisms in *Escherichia coli* O157:[H7] by Comparative Genome Resequencing Microarrays. Wei Zhang, Efrain Ribot, Eija Hyytia-Trees, and Bala Swaminathan. Foodborne and Diarrheal Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA.

Abstract: Shiga toxin-producing *E. coli* O157:[H7] are the predominant causative bacteria of bloody diarrhea and hemolytic-uraemic syndrome in the United States. Despite high clonality by MLST, genomes of *E. coli* O157:H7 have been demonstrated to be highly diverse by various DNA fragment-based genotyping methods, including PFGE and MLVA which primarily target insertions/deletions (indels), genomic rearrangements, or variable number of tandem repeats (VNTRs), respectively. A different approach using genomic sequence comparisons to identify single-nucleotide polymorphisms (SNPs) has emerged as a tool for subtyping *E. coli* O157:[H7]. In this study we use comparative genome resequencing microarrays to test potential SNP targets in 823 housekeeping and 376 conserved O157-specific genes (total 1.2 Mb, 20% of the genome) in ten *E. coli* O157 strains selected on the basis of geographic and genomic diversity. Approximately 166,000 oligonucleotide probes (29-mer) were designed, synthesized, and printed on mutation mapping microarray slides. Preliminary hybridization results have shown approximately 400 putative SNPs in the 1,199 genes among the test O157 strains in comparison to the reference of *E. coli* O157:[H7] strain Sakai. Interestingly, virulence genes seemed to be more conserved (less SNPs) than housekeeping genes, indicating recent heterogeneous acquisition of these virulence factors in *E. coli* O157 genomes. A second resequencing step is now being performed to accurately identify and locate these putative SNPs. Compared to indels and VNTRs, SNPs have a much lower mutation rate, and therefore, could be used as more reliable evolutionary markers to infer the genetic relatedness of *E. coli* O157 strains. Results from this study may provide useful markers for developing highly discriminatory next generation genotyping methods (e.g. multilocus SNP typing) for surveillance of *E. coli* O157:[H7]. Analysis of novel synonymous and non-synonymous SNPs identified in this study may also provide information relevant to the emergence, evolution, and pathogenesis of *E. coli* O157:[H7].

28. Molecular Subtyping of *Listeria monocytogenes* by Multi-Locus Variable Number Tandem Repeat Assay. Kate Volpe, Denise Griffin, Shadia Barghothi and Leslie Wolf North Carolina State Laboratory of Public Health, Raleigh, N.C.

Abstract: *L. monocytogenes*, the causative agent of listeriosis, is most commonly acquired through consumption of contaminated foods such as ready-to-eat meat or cheese products. PulseNet, the national molecular subtyping network for foodborne bacteria, is one key to early detection and identification of the strain(s) of bacterial species linked to an outbreak. Because *L. monocytogenes*, as well as other foodborne bacteria, have certain strains that are commonly linked to outbreaks, novel subtyping methods based on DNA sequences are required to further discriminate among these common strain types. Subtype-based surveillance in PulseNet laboratories is based on pulsed-field gel electrophoresis (PFGE). While this technique is currently state-of-the-art, inter-laboratory comparisons are difficult and require strict adherence to standardized protocols. Improving the detection of outbreaks and the discriminatory power of molecular subtyping methods is clearly a priority for food safety initiatives. Multi-locus variable number tandem repeat assay (MLVA) has proven to be a rapid and highly discriminatory subtyping tool for agents such as *Bacillus anthracis* (Keim et al). A novel MLVA for *L. monocytogenes* was developed for use in PulseNet laboratories based on these studies. Using tandem repeat finder software, 75 repeated sequences were identified in two *L. monocytogenes* strains (EGDe and 4b F2365). Twenty-seven of these loci were screened using SYBR green PCR and subsequent Fragment Analysis against a panel of 90 isolates. Sequencing was performed on a subset of isolates to verify the putative repeat. Nine of the loci

provide adequate diversity and are currently being evaluated for their ability to subtype the isolates into epidemiologically significant clusters. Preliminary data on 68 isolates has shown similar grouping to that of PFGE. Additional discrimination is seen with MLVA between some isolates with identical PFGE patterns. The data suggests that MLVA for *L. monocytogenes* is at least as, if not more, discriminatory than PFGE. Because MLVA is a high throughput screening method that is fairly inexpensive, easy to perform, rapid and reliable, it may be better suited to inter-laboratory comparisons during epidemiological investigations of foodborne illness.

29. Comparison of Multiple-Locus Variable Number Tandem Repeat Analysis, Pulsed-Field Gel Electrophoresis and Phage Typing for *Salmonella enterica* Serotype Enteritidis. **D. Boxrud**[1], K. Pederson-Gulrud[1], C. Medus[1], J. Besser[1], E. Lyszkowicz[2], J. M. Bartkus[1]; [1]Minnesota Department of Health, Minneapolis, MN, [2]Centers For Disease Control and Prevention, Atlanta, GA.

Abstract: *Salmonella enterica* Serotype Enteritidis (SE) has emerged as the second most common *Salmonella* serotype from humans in the US over the last 20 years. Methods previously used for subtyping SE have shown limited diversity which restricts the usefulness of the method for detecting outbreaks. Pulsed-field gel electrophoresis (PFGE) using *Xba*1 and *Bln*1, phage typing (PT), and a 10 locus Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) method developed at the Minnesota Department of Health were used to analyze 153 SE isolates recovered from Minnesota residents during the years 1998-2003. All cases were interviewed about potential exposures and recent travel history. The study included 113 isolates from sporadic cases and 40 isolates from cases from 4 outbreaks. Among the sporadic case isolates, there were a total of 58 MLVA types, 33 PFGE types and 16 PTs. The Simpson's diversity index, a measure of discriminatory power, was 0.98 for MLVA, 0.84 for PFGE and 0.85 for PT. MLVA types were found within the most common PFGE (SE1B1 and SE11B6) and phage types (PT 4, PT 8 and PT 13a). PFGE types SE11B6 (n=29) and SE1B1 (n=34) were divided into 12 and 19 MLVA types respectively. Phage types PT 4 (n=29), PT 13a (n=26) and PT 8 (n=17) were divided into 14, 17 and 11 MLVA types, respectively. PFGE was unable to differentiate between PT8 and PT13a, but these phage types were segregated and further differentiated by MLVA. Conversely, there were instances where PFGE or PT was able to differentiate isolates with the same MLVA type. Two of the most common MLVA types (MSE11 and MSE9) were further discriminated by both PFGE and phage typing. MLVA type MSE11 (n=6) contained 4 PFGE types and 5 PTs. MLVA type MSE9 (n=17) contained 5 PFGE types and 4 PTs. A group of sporadic isolates that clustered by MLVA (n=38) but not by PFGE was associated with foreign travel shortly preceding illness (OR: 62.7; 95% CI: 15.7 to 288.3; $p<0.001$) compared to isolates with MLVA types not in this cluster. Within each of the 4 outbreaks, all isolates were identical by MLVA, PFGE and PT, except 1 isolate that had a different PT than the outbreak PT. A different MLVA type was associated with each outbreak. Two of the outbreaks were associated with a single PFGE type and all 4 outbreaks were associated with a single PT. Among the sporadic isolates, subtypes matching the outbreak strains occurred for 11/113 (9.7%) by MLVA, 35/113 (31%) by PFGE, and 26/113 (23%) by PT. Sporadic isolates matching outbreak strains represent unrecognized outbreak-associated cases, endemic subtypes, or strains falsely classified due to lack of subtype resolution. MLVA appears to provide greater discriminatory power than either PFGE or phage typing. All outbreak-associated isolates were properly classified by MLVA, and a cluster of isolates associated with foreign travel was identified by MLVA but not PFGE or PT. These data suggest an important potential role for MLVA in SE subtyping.

30. Comparison of MLVA and PFGE for Subtyping Serogroup C *Neisseria meningitides.* J. O'Connell, P. Killoran, and W. Probert. Microbial Diseases Laboratory, California Department of Health Services, 850 Marina Bay Parkway, Richmond, CA 94804

Abstract: Molecular subtyping is of paramount importance to the recognition of outbreaks of meningococcal disease caused by serogroup C *Neisseria meningitidis.* We describe the application of Multilocus Variable Number Tandem Repeat Analysis (MLVA) for the molecular subtyping of *N. meningitidis* and compare its performance to that of the current gold standard of strain typing, Pulsed Field Gel Electrophoresis (PFGE). For MLVA, a multiplex PCR assay targeting five VNTRs was developed and evaluated using a panel of sporadic and outbreak-associated serogroup C, *N. meningitidis* isolates. The amplified products were separated by agarose gel electrophoresis and the MLVA patterns analyzed using Bionumerics software following standard PulseNet procedures. MLVA was highly reproducible and provided a result turnaround time of 6 hours. Overall, the discriminatory power of MLVA was equal to that of PFGE. The utilization of MLVA for subtyping *N. meningitidis* isolates provides a more rapid and safer alternative to PFGE for identifying outbreaks of meningococcal disease and may provide Public Health officials with information essential to the prevention of further cases of this devastating disease.

31. *Escherichia coli* O157 Unique PFGE Pattern (UPP) Isolate Archive. Nancy Garrett[1], Eija Hyytiä-Trees[1], Molly Joyner[1], Jennifer Kincaid[1], Evangeline Sowers[1], Efrain Ribot[1], and the PulseNet Participating Laboratories. [1]CDC, FDDB, Atlanta, GA.

Abstract: A unique PFGE pattern (UPP) is defined as a pattern that is different from any other PFGE pattern in the organism-specific database. The number of unique PFGE patterns and the level at which they differ from each other reflect the amount of diversity within particular collection of isolates. In 2004, the CDC PulseNet Methods Development and Validation Laboratory started requesting *E. coli* O157 UPP isolates from the PulseNet participating laboratories. The objective of the project was to store all UPP isolates in a centralized location in order to have them available for future characterization and research needs. A total of 861 cultures from 30 laboratories were received by January 2005. Each culture was checked for purity, sorbitol-fermentation status and tested by PCR for the Shiga toxin genes (stx_1, stx_2) and for the 1 bp mismatch in the ß-glucuronidase gene (*uidA*) that is specific for *E. coli* O157:[H7} serotype. Atypical isolates were sent to the CDC National *E. coli* Reference Laboratory for biochemical identification and serotyping. About 20 % of the isolates were re-tested by PFGE and the patterns were compared against the original submission in the National Database. The majority of the isolates were positive for both Shiga toxin genes (60 %) or for the stx_2 gene only (27%). Of the 56 atypical isolates identified, 31 were fermenting sorbitol. The atypical isolates characterized by the Reference Laboratory so far are either mixed cultures, non-O157 STEC, O157 isolates of H-types other than H7, non-pathogenic *E. coli* or species other than *E. coli*. Of the 163 isolates that were re-tested by PFGE, 136 (83.4 %) had patterns matching the original submission. Sub-optimal gel quality in the original submission, band marking inconsistencies and mix-up of isolates in the submitting laboratory were main reasons why some of the re-tested patterns did not match the original submissions. The UPP project has already proved to be a useful tool for checking the accuracy of the data in the National Database. The relatively high number of atypical isolates emphasizes the importance of correct identification and serotyping of isolates. In many cases, the reproducibility of the PFGE patterns could be improved by particularly focusing on high gel quality.

32. Use of thiourea and pulsed-field gel electrophoresis to resolve DNA fingerprint patterns from previously untypeable strains of *Clostridium perfringens* implicated in a foodborne outbreak at an assisted living facility. **ELISE B. SMITH***, KELLY D. FELKEY, DENISE M. TONEY. Commonwealth of Virginia, Department of General Services, Division of Consolidated Laboratory Services.

Abstract: *Clostridium perfringens* is a common cause of foodborne illness each year. The ability to identify and cluster strains through genotypic methods has proven useful to the epidemiologic investigation of *C. perfringens* outbreaks since this organism is often present as normal gastrointestinal flora. Pulsed-field gel electrophoresis (PFGE) is routinely used for genotypic discrimination of bacterial strain types; however, many *C. perfringens* strains resolve poorly by PFGE due to DNA degradation most likely attributed to endogenous bacterial nucleases. On November 3, 2004, the Division of Consolidated Laboratory Services was notified of an outbreak of gastroenteritis at an assisted living facility. Clinical specimens were collected and cultured for the presence of *C. perfringens*. *Clostridium perfringens* isolates from each positive specimen were tested for the presence of enterotoxin (cpe) production and genotyped by PFGE using *Sma* I digestion. Initial PFGE analysis failed to yield resolvable DNA fingerprint patterns. Alterations in the cell culturing protocol and the addition of 50µM thiourea to the electrophoresis buffer allowed for complete resolution of DNA fingerprint patterns and identification of a common outbreak strain type in 7 of 8 specimens. Furthermore, PFGE demonstrated that both enterotoxin positive and enterotoxin negative isolates possessed indistinguishable DNA fingerprint patterns, suggesting a common outbreak strain type. These results demonstrate the importance of PFGE fingerprinting for the detection and characterization of *C. perfringens* strains implicated in foodborne disease outbreak investigations.

33. Expanding the net: from Foodborne to Vectorborne PFGE typing of *Yersinia pestis* and *Francisella tularensis.* **Kristy Kubota**, Kate Lesciotto, Marty Schriefer. Division of Vector-borne Infectious Diseases, CDC, Fort Collins, CO.

Abstract: *Yersinia pestis* and *Francisella tularensis* are the etiologic agents of the zoonotic diseases plague and tularemia, respectively. Small rodents and their blood feeding ectoparasites, mainly fleas and ticks, are largely responsible for natural disease maintenance and occasional transmission to humans. In humans, these diseases often cause significant morbidity and mortality. Plague is endemic in the Southwestern portion of the US and yearly human incidence has ranged from less than 5 to 40 cases over the last 20 years. Tularemia, in contrast, is broadly distributed across the country with several hundred cases reported yearly and focal concentrations of disease in the Midwest and east-coast. Of greater recent concern than natural disease however, is the potential use of these agents as bioweapons. In order to better monitor both natural and potentially introduced disease in the US, the Division of Vector-borne infectious Diseases in collaboration with the Division of Bacterial Mycotic Diseases of the Centers for Diseases Control and Prevention has expanded PulseNet to PFGE typing of clinical and environmental isolates for plague and tularemia.

PFGE protocols and scripts have been developed for *Y. pestis* and a database with more than 750 entries and over 280 unique profiles has been compiled. This database is in the production phase for beta testing with external partner laboratories. *F. tularensis* protocol, utilizing two enzymes to improve discrimination within type A subspecies, is also in development and protocols enabling distinction of type B *F. tularensis* isolates are under evaluation. Additionally, two PulseNet conferences have been added to the WebBoard internet site and wet bench training for the Utah area laboratories in *Y. pestis* PFGE typing will be conducted later this summer.