

10th Annual PulseNet Update Meeting Abstracts

(National Foodborne Epidemiologists Meeting abstracts begin on page 12)

1. PulseNet Europe – A European Network for Molecular Surveillance of

Foodborne Infections. Susanna Lukinmaa on behalf of PulseNet Europe partners.

Abstract: PulseNet Europe (PNE) is a network of food, public health and veterinary laboratories dedicated to molecular surveillance of foodborne infections in Europe. PNE is currently funded until November 2006 by the FP6 network of excellence Med-Vet-Net, a virtual European Zoonosis Centre (<http://www.medvetnet.org/>).

The network's main aim is to establish a surveillance database able to detect and investigate possible outbreaks of *Salmonella*, VTEC and *Listeria monocytogenes*. The PNE database is built in the BioNumerics server/client format and the server is maintained by the Health Protection Agency, Colindale, England (HPA). At present a PNE database is operating on a trial basis and is expected to be fully functional by May 2006.

The typing method presently used is pulsed-field gel electrophoresis (PFGE) in accordance with the PulseNet USA protocol. Six trained PNE curators will be responsible for the quality of the data in the databases including naming and confirmation of the PFGE profiles and central cluster detection. Alerts will be sent to the partners through the Web-based communication system (PNE forum) that has been set up to streamline and accelerate communication within the network.

The collaborating partners come from 59 institutes from 30 countries. With additional funding from Med-Vet-Net WP2, the first PFGE laboratory and analysis training course was organised at HPA in February 2006 with 15 participating partners. A quality assurance system has been established and the partners will be certified based on their ability to produce images which can be directly submitted to the database. At a later stage the quality of gels and skills for analysis will be tested with Proficiency Testing. A Memorandum of Understanding is presently being drafted to define the rules for collaboration allowing exchange of information and molecular typing data between PNE partners but also with PulseNet USA.

It is expected that when PulseNet Europe is fully operational and participating countries submit DNA fingerprinting data to the central database, the detection and control of foodborne outbreaks will be significantly improved in Europe.

2. PulseNet *E. coli* O157:H7 National Database Year in Review, 2005. M. Joyner, P. Gerner-Smidt, K. Hise.

Abstract: This poster presents a summary of activity within the *E. coli* National Database in 2005. It gives an overview of the most common patterns seen in 2005 and descriptions of the year's major outbreaks.

3. Shiga toxin-producing *Escherichia coli* O157 (STEC O157) Unique PFGE

Pattern (UPP) Isolates from 2004. Nancy Garrett, Efrain Ribot, Molly Joyner, Kathy Greene, Eija Hyytiä-Trees, and the PulseNet Participating Laboratories

Abstract: A PulseNet unique PFGE pattern (UPP) is defined as a pattern that is different from any other PFGE pattern in the organism-specific national database. The number of unique PFGE patterns and the level at which they differ from each other

reflect the amount of diversity within a particular collection of isolates. In 2004, the CDC PulseNet Methods Development and Validation Laboratory created the STEC O157 UPP archive program. 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. During the first year of the program (2004), UPP cultures from 1998 through 2003 were requested and processed. In 2005, the UPP cultures from 2004 were requested resulting in a total of 320 culture submissions to CDC. 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 β-glucuronidase gene (*uidA*) that is specific for STEC O157 serotype. A typical STEC O157 culture was defined as negative for sorbitol fermentation, positive for toxin production and the *uidA* mismatch by PCR or for the O157 antigen by latex and PCR. About 25 % of the cultures were re-tested by PFGE and the patterns were compared against the original submission in the National Database. The majority of the cultures were positive for both Shiga toxin genes (51.1 %) or for the *stx*₂ gene only (32.9 %). A total of 32 cultures not fitting in the definition for a typical culture were identified. Fifteen of these cultures were sent to the CDC Epidemic Investigations and Surveillance Laboratory (EISL) for further identification / purification and serotyping. They were either mixed cultures, non-O157 STEC, O157 isolates of H-type other than H7, or non-pathogenic *E. coli*. Of the 82 isolates that were re-tested by PFGE, 66 (80.5 %) had patterns matching the original submission. Mix-up of isolates in the submitting laboratory, possible mutations and sub-optimal gel quality in the original submission were main reasons why some of the re-tested patterns did not match the original submissions. The UPP project has proven to be a useful tool for checking the accuracy of the data in the National Database. A relatively high increase in the proportion of atypical cultures compared to the previous year (10 % vs. 6.5 %) was detected. This emphasizes the importance of correct identification and serotyping of isolates. Use of SMAC medium for isolation and purity check, and serotyping both O and H antigens is therefore highly recommended.

4. The PulseNet PFGE protocol for *Vibrio vulnificus*: An Update. Michele Bird, K. Cooper, E. Ribot and C. Bopp.

Abstract: *V. vulnificus* is a halophilic bacterium that naturally inhabits warm seawaters. Persons become infected with *V. vulnificus* by consuming raw or undercooked shellfish, particularly oysters from the Gulf Coast, or by direct contact of broken skin with warm brackish water containing *V. vulnificus*. A third of cases are fatal and many others result in life-threatening septicemia or amputation of a limb. *V. vulnificus* is classified into three biogroups, but only Biogroups 1 and 3 are known to cause human illness. Strains isolated from the United States are of biogroup 1. Biogroup 3 *Vibrio vulnificus* infections have only been reported from Israel. Human infections of *V. vulnificus* are typically sporadic and outbreaks are rarely identified. Subtyping, in combination with surveillance data and, when available, seafood traceback data could be very useful in linking cases with a common source and tracking the persistence of this pathogen in the environment. This work provides an update on the development of a PulseNet standardized PFGE protocol for subtyping *V. vulnificus*. The establishment of this method would provide a reliable

means to compare and catalog strains of this species in the United States and worldwide.

- 5. Towards an International PulseNet Standardized Pulsed-field Gel Electrophoresis (PFGE) Protocol for *Vibrio parahaemolyticus*. Cindy Luey** on behalf of The PulseNet International *Vibrio parahaemolyticus* Work Group.
Abstract: *Vibrio parahaemolyticus* is an important foodborne pathogen globally, and especially in the South East Asian region. Subspecies genotyping by pulsed-field gel electrophoresis (PFGE) is useful for outbreak investigations. Because of the global scale of spread of this organism, as well as public health implications of large foodborne outbreaks, it is critically important to have an internationally validated and accepted standardized *V. parahaemolyticus* PFGE protocol. Such a protocol will enable investigation results to be meaningfully compared across countries that involve different public health laboratories using PFGE to subtype this organism.
- This poster describes the development and ongoing evaluation and validation of a standardized pulsed-field gel electrophoresis (PFGE) protocol for *Vibrio parahaemolyticus*. This project is a collaborative effort between the following international PulseNet Participating public health laboratories:
- (1) International Centre for Diarrhoeal Diseases Research, Bangladesh;
 - (2) Public Health Laboratory Centre, Hong Kong;
 - (3) National Institute of Cholera and Enteric Diseases, India;
 - (4) National Institute of Infectious Diseases, Japan;
 - (5) National Institute of Health, Thailand; and
 - (6) Centers for Disease Control and Prevention, PulseNet USA – PulseNet Methods Development and Validation Laboratory
- 6. PulseNet *Campylobacter* National Database Year in Review, 2005. J. Lockett, M. Adams.**
Description: This poster will present a review of statistics, notable outbreaks, and common patterns for the *Campylobacter* database as of December 2005.
- 7. PulseNet *Shigella* National Database Year in Review, 2005. DL Jennings.**
Description: This poster will give a summary of the *Shigella* database, statistics on submitting laboratories, top five patterns, and serotype distribution as of December 2005.
- 8. The Role of Pulsed-Field Gel Electrophoresis in Investigating a Missouri *Shigella sonnei* Outbreak. Jason Herstein, Stephanie Barnhart, Amy Pierce, Jo Ann Rudroff, C. Jon Hinkle, Stephen Gladbach.**
Abstract: Pulsed-Field Gel Electrophoresis of *Shigella sonnei* has been used as a successful tool to link outbreaks to specific sources. The continuing northwestern Missouri shigellosis outbreak that began in the late spring of 2005 produced a large number of isolates that were submitted to the MO SPHL for confirmation and subtyping. There were pattern similarities between isolates that were epidemiologically linked to the ongoing outbreak, but there were no indistinguishable

patterns linked to a specific source. PFGE data from May 2005 to January 2006 was reexamined in conjunction with epidemiologic results. The data suggests that as the duration of the outbreak progresses the increasing variability of patterns decreases the value of the PFGE information.

9. PulseNet *Salmonella* National Database Year in Review, 2005. AM Camba, JL Lockett, NJ Patel, MS Van Duyne.

Description: This poster will visually describe the National *Salmonella* database for the year of 2005. The information includes statistics on submitting laboratories, distribution of samples, top five serotypes, notable outbreaks of 2005, and states goals of 2006.

10. Public Health in Action: A multi-agency response to a *Salmonella* Typhimurium outbreak in San Diego County, California. Cathy Adams, Mark Janowski, Andrea Mikolon, Hailu Kinde, Alfonso Rodriguez.

Abstract: During 2004, San Diego County had an on-going persistent outbreak of *Salmonella* Typhimurium that a case control investigation revealed was associated with the consumption of fresh Mexican style cheese. The cheese (primarily queso fresco) was being purchased from local street vendors who operate in neighborhoods that are predominantly Mexican-American. The vendors sell their products, for which a huge demand exists within the community, usually out of the trunk of their vehicle. In response to this outbreak, several agencies came together to engage in this sensitive and cultural issue of selling and consuming unpasteurized cheese products. The Queso Fresco Committee spent four days at the San Ysidro border crossing and conducted a voluntary survey in order to determine how much unregulated cheese products were crossing the border.

11. A Tale of Two Restaurants: Use of MLVA to Expand the Epidemiological Investigation of a California *Salmonella* Enteritidis Outbreak. J. O'Connell, P. Killoran, K. Schrader, J. Yuan and W. Probert.

Abstract: *Salmonella* Enteritidis is one of California's most common serotypes. In July 2005, the MDL had what we thought were two outbreaks in two Mexican restaurants located across the bay from each other. PFGE results on the initial isolates from these two restaurants showed that the XbaI and BlnI patterns were the same. The SE PFGE XbaI pattern turned out to be XbaI pattern JEGX01.0012 (pattern 12), a previously rare pattern that had only been seen in raw almonds. Since the number of SE cases statewide in July of 2005 was up two and a half times over that for July 2004, we decided to test all of the isolates with date collected in July to see how many were pattern 12. After setting up 86 isolates for PFGE with the primary enzyme (and 54 second enzyme PFGE's), we developed a MLVA test for pattern 12 based on Ramisse et al.'s genomic sequence analysis of SE. Development and validation of this test allowed more isolates to be tested and much more rapid screening of isolates which helped to further define the outbreak.

12. Next Generation Molecular Subtyping of *Salmonella* by Multiple Locus Variable Number Tandem Repeat Assay. Kristin Pederson.

13. Multiple locus variable number of tandem repeats: Improved surveillance and outbreak detection of human *Salmonella* Typhimurium infections. Mia

Torpdahl, Gitte Sørensen, Bjørn-Arne Lindstedt and Eva Møller Nielsen.

Abstract: In Denmark there is an extensive coordinated surveillance of *Salmonella* in humans, animals and food. As part of the routine surveillance of *S. Typhimurium*, isolates from all confirmed human infections are real-time typed using PFGE, phage typing and antimicrobial resistance profiles. From December 2003 to December 2005, only a few phage types accounted for most infections and within the most frequently isolated phage types, DT104, DT120 and DT12, approximately 65% of all isolates were assigned to a single PFGE type. We therefore included multiple locus variable number of tandem repeats analysis (MLVA) typing in the routine surveillance in the same time period in order to evaluate the discriminatory ability and the usefulness in cluster detection and outbreak investigations of MLVA. In total, 1019 isolates were typed and separated into 148 PFGE types and 373 MLVA types. Several unique MLVA clusters were found that were supported either by other typing data or by epidemiological data. Several of the clusters were also linked to a common source by MLVA typing of food and animal isolates or by epidemiological data. A few Norwegian cases had the same MLVA type as seen in clusters in Denmark and interviews revealed that all such patients had been traveling to Denmark. In conclusion, we found that MLVA improved the surveillance of human *S. Typhimurium* infections in Denmark. Compared to PFGE and phage typing, MLVA was faster to perform, easier to interpret and more discriminatory. Several possible outbreaks were detected that otherwise might not have been detected or solved and MLVA might be of value in local, national and international surveillance of *S. Typhimurium* infections.

14. PulseNet *Listeria* National Database Year in Review, 2005. NJ Patel.

Description: This poster will present a summary of the *Listeria* database as of December 2005, a description of notable *Listeria* outbreaks of 2005, and states goals of 2006.

15. Next Generation Molecular Subtyping for *Listeria monocytogenes* by Multiple Locus Variable Number Tandem Repeat Assay. Kate Volpe, Denise Griffin and Leslie Wolf.

Abstract: The Multiple Locus Variable Number Tandem Repeat (VNTR) Assay (MLVA) is a rapid and high throughput genotyping method based on DNA sequence that can be implemented in PulseNet laboratories to replace or complement existing protocols. While PFGE is currently state-of-the-art, inter-laboratory comparisons can be difficult and require strict adherence to standardized protocols. MLVA utilizes capillary electrophoresis to accurately size PCR amplified DNA fragments. These fragments are designed around VNTRs found throughout the genome. For every isolate, fragment size is used to determine the number of repeats in each locus tested. These repeats have been found to be highly variable amongst different strains. Strain clustering is based on repeat number at multiple loci, making data portability seamless between laboratories. Using the MLVA, 43 of 75 loci identified in the *L. monocytogenes* genome have been screened for diversity. Currently, 12 loci are

being evaluated for their ability to subtype a panel of 180 isolates into epidemiologically significant clusters. MLVA subtyping using 8 loci on 41 epidemiologically linked isolates and 11 isolates from unknown origin correlated well with PFGE results. To improve subtyping ability four additional loci are under investigation. This research has shown that the MLVA is fairly inexpensive, easy to perform, rapid and reliable; furthermore, it may be better suited to inter-laboratory comparisons during epidemiological investigations of foodborne disease outbreaks.

16. Comparison of a Multiplex PCR Assay and Conventional Serotyping for Seroclassification of *Listeria monocytogenes* Isolates: 2005 Update. Lewis M. Graves, Robin Broeker, Nancy Garrett, and Bala Swaminathan.

Abstract: A recently validated multiplex PCR assay was evaluated against conventional serotyping using 577 *Listeria monocytogenes* isolates. Serotyping is useful as a first-level discriminator and availability of serotype information on clinical isolates allows investigators to exclude cases that are not part of an outbreak under investigation. The multiplex PCR assay is designed to cluster *L. monocytogenes* isolates into four major groups (Gp) (Gp 1: serotypes 1/2a, 3a; Gp 2: 1/2b, 3b, 7; Gp 3: 1/2c, 3c; Gp 4: 4b, 4d, 4e). *Listeria* isolates that do not group into one of the four major groups should group in a species-specific group that is designated “Gp-L”. During the course of this study, 3 undefined profiles of multiplex PCR Gp 4 were identified. The *L. monocytogenes* isolates used for this study included all sporadic clinical *L. monocytogenes* surveillance isolates received in the *Listeria* Surveillance laboratory between January 6, 2005 and December 31, 2005. The concordance between the two methods for serotypes 1/2a, 1/2b, and 4b was 96%, 94%, and 84%, respectively.

17. Using Restriction Endonuclease *Sma*I for Subtyping *Listeria monocytogenes* by Pulse-Field Gel Electrophoresis: A Preliminary Study. Robin Broeker and Lewis Graves.

Abstract: *Listeria monocytogenes* is the causative agent of listeriosis. Pulsed-field gel electrophoresis (PFGE) provides a tool for detecting and investigating listeriosis outbreaks. The *Listeria* PFGE protocol uses the restriction endonucleases (RE) *Asc*I and *Apa*I. In this study, RE *Sma*I was evaluated to determine its utility for subtyping *L. monocytogenes* by PulseNet participating laboratories. To accomplish this, PFGE running conditions for *Sma*I were established. A reference standard pattern with the universal standard *Salmonella* ser. Braenderup was created. Then, 19 *L. monocytogenes* isolates (4 from the *Listeria* certification set, 8 from the 1998-99 outbreak, 3 from the 2002 outbreak, and 4 isolates from sporadic cases of listeriosis in 2003) were run with *Sma*I. All isolates had discernible bands within BioNumerics. For the same isolate, *Sma*I produced more DNA fragments in the evaluation range than *Asc*I or *Apa*I. This preliminary study suggests that *Sma*I may generate greater discrimination among *Listeria* isolates than *Apa*I.

18. Using a database of pulsotypes from food isolates to solve clusters of clinical cases. Lyn O’Reilly.

Abstract: Perth, a city of 2 million people, is 2000km from the nearest capital city. It has developed many smallgoods producers. PathWest is responsible for food and water testing and performs diagnostic tests for 24 metropolitan and regional branches of PathWest throughout Western Australia.

The molecular typing laboratory, in addition to keeping databases on isolates from clinical cases, has two databases containing PFGE patterns from food isolates. The food databases are for *Listeria* and *Salmonella*. Clinical isolates then can be compared to isolates contained in the food database.

Observations from the *Listeria* database indicate that groups of foods with an indistinguishable pulsotype either are different products from the same factory or are the same primary food product from different factories. It also appears that factories maintain a certain pulsotype over many years. There have been several successful investigations and outcomes using this database that was initiated in 2000.

Three clusters of *Salmonella* occurred in 2005. No food isolate was found on the database from historical isolates that matched a big cluster of *Salmonella* Oranienberg clinical cases. However continual investigation yielded a food isolate with an indistinguishable pattern. A food collected just before the outset of a clinical cluster of *Salmonella* Virchow that contained *S. Virchow* could be eliminated from that investigation as it had a different pulsotype. A food isolate pulsotype was found on the database for *Salmonella* Bovismorbificans that gave the environmental officers a focus for collection of samples.

19. Characterization *Salmonella enterica* serovar Heidelberg from Retail Meat

Samples: National Antimicrobial Resistance Monitoring System (NARMS): 2002-2004. S. Zhao, D. G. White, S. L. Friedman, A. Glenn, S. L. Ayers, J. W. Abbott, S. Hubert, E. Hall-Robinson, H. Harbottle, R. D. Walker and P. F. McDermott.

Background: *Salmonella enterica* serovar Heidelberg is frequently associated with foodborne illness in humans and is commonly isolated from poultry and its derived meats. A recent upsurge in antimicrobial resistance in this serovar has been recognized. There are few data on the prevalence, antimicrobial susceptibility, and genetic diversity of *S. Heidelberg* isolates in retail meats. **Methods:** We compared the prevalence of *S. Heidelberg* in a sampling of 10,745 meats, including chicken breast (n=2,685), ground turkey (n=2,664), ground beef (n=2,708) and pork chops (n=2,688) collected during 2002-2004 for the NARMS retail meat surveillance program. Isolates were analyzed for antimicrobial susceptibility and compared genetically using pulsed-field gel electrophoresis (PFGE). **Results:** A total of 154 *S. Heidelberg* isolates were recovered, representing 22.4% of all salmonella serovars from retail meats. Among the 154 isolates, 90 (58.4%) from ground turkey, 58 (37.7%) from chicken breast and 6 (3.9%) from pork chop; no *S. Heidelberg* was found in ground beef. Ninety-four (61%) of the isolates were resistant to at least one of the 16 antimicrobial agents tested and twenty-five (16.2%) of the isolates were resistant to ≥ 5 antimicrobials. Five isolates (3.2%) were resistant to ≥ 9 antimicrobials, which were all recovered from ground turkey meat. The highest rates of resistance from poultry meat isolates were observed for tetracycline (39.86%) followed by streptomycin

(38.51%), sulfamethoxazole (25%), gentamicin (22.97%), and kanamycin (22.30%), ampicillin (16.89%), cephalothin (8.1%), cefoxitin (6.76%), ceftiofur (6.76%), amoxicillin-clavulanic acid (5.41%), chloramphenicol (2.03%), and nalidixic acid (0.68%). All isolates were susceptible to amikacin, ceftriaxone, ciprofloxacin and trimethoprim/sulfa. PFGE with *XbaI* and *BlnI* generated 61 patterns for the 154 isolates. Certain clones were widely dispersed in different types of meats and meat brands from different store chains in all three years. **Conclusions:** These data indicate that *S. Heidelberg* is a common serovar in retail poultry meats, and includes widespread clones of multidrug-resistant strains.

20. Antimicrobial resistance and genetic relatedness among *Salmonella* from retail foods of animal origin: NARMS retail meat surveillance. S. Zhao, P.F.

McDermott, S. Friedman, J. Abbott, S. Ayers, A. Glenn, E. Hall-Robinson, S.K. Hubert, H. Harbottle, R.D. Walker, T. M. Chiller and D.G. White.

Abstract: The National Antimicrobial Resistance Monitoring System (NARMS) retail meat component is a collaborative effort between FDA, CDC, and FoodNet, which monitors trends in antimicrobial susceptibility and genetic relatedness among select foodborne bacterial pathogens and commensal organisms. *Salmonella* isolates were recovered from a monthly sampling of chicken breasts, ground turkey, ground beef and pork chops purchased from selected grocery stores in 6 participating FoodNet sites (CT, GA, MD, MN, OR, TN) in 2002 and an additional 2 sites in 2003 (CA and NY). Retail meat samples tested increased to 3,533 in 2003 as compared to 2,513 in 2002. Overall, six percent of 6,046 retail meat samples (N=365) were contaminated with *Salmonella*, the bulk recovered from either ground turkey (52%) or chicken breast (39%). *Salmonella* isolates were serotyped and susceptibility tested against a panel of 16 antimicrobial agents using CLSI/NCCLS broth microdilution methods. *S. Heidelberg* was the predominant serotype identified (23%), followed by *S. Saintpaul* (12%), *S. Typhimurium* (11%), and *S. Kentucky* (10%). Overall, resistance was most often observed to tetracycline (40%), streptomycin (37%), ampicillin (26%), and sulfamethoxazole (25%). Twelve percent of isolates were resistant to cefoxitin and ceftiofur, though only 1 isolate was resistant to ceftriaxone. All isolates were susceptible to amikacin and ciprofloxacin; however, 3% of isolates were resistant to nalidixic acid and were recovered primarily from ground turkey samples (n=11/12). All *Salmonella* isolates were analyzed for genetic relatedness using PFGE patterns generated by digestion with *XbaI* or *XbaI* plus *BlnI*. PFGE fingerprinting profiles showed that *Salmonella*, in general, were genetically diverse with a total of 175 *XbaI* PFGE profiles generated from the 365 isolates. PFGE profiles showed good correlation with serotypes and in some instances, antimicrobial resistance profiles. Results demonstrated a varied spectrum of antimicrobial resistance and genetic relatedness, including several multidrug resistant clonal groups among *Salmonella* isolates, and signifies the importance of sustained surveillance of foodborne pathogens in retail meats.

21. Characterization of Antimicrobial Resistance of *Salmonella* Isolates Recovered From Diseased Animals. S. Zhao, P. F. McDermott, D. G. White, S. Qiayumi, S. L.

Friedman, J. W. Abbott, A. Glenn, S. L. Ayers, P. K. Post, W.H. Fales, R. B. Wilson, C. Reggiardo, and R. D. Walker.

Abstract: A total of 380 *Salmonella* isolates representing 47 serotypes recovered from diseased animals in year 2002-2003 were characterized using antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE) and examined for the presence of class-1 integrons and the *bla*_{CMY} gene. Overall, 82.4% of isolates were resistant to at least one antimicrobial, 69.7% to ≥ 3 , and 15.5% to ≥ 9 antimicrobials. Many isolates exhibited resistance to tetracycline (78.2%) follow by streptomycin (72.6%), sulfamethoxazole (67.6%), ampicillin (53.7%), chloramphenicol (37.1%), kanamycin (36.8%), cephalothin (29.2%), amoxicillin-clavulanic acid (20.3%), cefoxitin (17.6%), ceftiofur (17.4%), gentamicin (12.6%), trimethoprim-sulfmethoxazole (8.7%), nalidixic acid (3.9%), ceftriaxone (2.1%), and amikacin (0.5%). All isolates were susceptible to ciprofloxacin. Swine isolates had the highest resistance rate; 91.5% were resistant to at least one antimicrobial. The resistance rates of *Salmonella* isolates from turkey, cattle, chicken, and equine were 90.6%, 77%, 68%, and 20%, respectively. However, more than one third of cattle isolates (34.9%) were resistant to ≥ 9 antimicrobials, whereas only approximately 10% equine or turkey isolates showed resistant to ≥ 9 antimicrobials. Antimicrobial resistance of the *Salmonella* isolates was also related to serotype. A large percentage of isolates belonging to *S. Uganda* (100%), *S. Agona* (78.6%), and *S. Newport* (62%) showed resistant to ≥ 9 antimicrobials, compared to *S. Heidelberg* (11.4%) and *S. Typhimurium* (7%). Class-1 integrons were commonly detected (43.2 %), containing *aadA*, *aadB*, *dhfr*, *cmlA* and *sat* alone or in combinations. PFGE showed considerable genetic diversity across all isolates, however, several multiple drug resistance clones were widely spread among animals.

22. Characterization of *Campylobacter* recovered from NARMS Retail Meats by Pulsed-Field Gel Electrophoresis. P. Cullen, S. Friedman, J. Abbott, L. English, A. Stearns, P. McDermott, D. White, R. Walker, S. Zhao, and the NARMS Working Group.

Abstract: *Campylobacter* are ubiquitous in the animal production environment and are a leading cause of foodborne illness. The objective of this study was to investigate the prevalence and genetic relatedness of *Campylobacter* spp. recovered from retail meats. In 2004 ten NARMS FoodNet sites collected 4,699 retail meats, including chicken breast (n=1172), ground turkey (n=1165), pork chops (n=1176) and ground beef (n=1186). A total of 721 *Campylobacter* isolates were recovered, with the highest recovery rate occurring in chicken breast (60.2%, n=706), followed by ground turkey (<1%, n=12) and pork chops (<1%, n=3). No *Campylobacter* was found in ground beef. Isolates previously identified to a species level (517 *C. jejuni* and 204 *C. coli*) were further characterized by pulsed-field gel electrophoresis (PFGE). *Sma*I restriction yielded 214 unique *C. jejuni* patterns and 103 *C. coli* patterns. Isolates with indistinguishable *Sma*I patterns (*C. jejuni*=93 patterns; *C. coli*=41 patterns) were further analyzed with *Kpn*I. Analysis of *Sma*I in conjunction with *Kpn*I identified several clonal profiles, including 12 *C. jejuni* recovered from 4 states (CA, CT, MN and OR) over a three month time span. Isolated DNA from three *C. jejuni* that did not cut with *Sma*I were successfully digested with *Kpn*I, yielding

identical patterns. Conversely, ten isolates resistant to *KpnI* digestion generated 4 distinct patterns when cut with *SmaI*. In conclusion, while substantial genomic diversity exists within *Campylobacter spp.*, the use of two enzymes in performing a PFGE analysis allows for the detection of clones that are dispersed throughout various temporal and geographical ranges.

23. Characterization of Salmonella enterica serovar Agona Slaughter Isolates from the Animal Arm of the National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS): 1997 through 2003. Aphrodite Douris, Paula J. Fedorka-Cray and Charlene R. Jackson.

Abstract: A total of 499 *Salmonella enterica* serovar *Agona* isolates were assayed for antimicrobial susceptibility and subtyped using pulsed field gel electrophoresis. All isolates were collected as part of the animal arm of the National Antimicrobial Resistance Monitoring System - Enteric Bacteria (NARMS) from slaughter and processing samples at federally inspected plants. Isolates originated from cattle, swine, chicken and turkey samples for the years 1997 through 2003. *Salmonella Agona* isolates exhibited increased resistance to 6 of the 19 antimicrobials tested: amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, and chloramphenicol. Although all isolates were susceptible to ciprofloxacin, an increase in resistance to the quinolone, nalidixic acid was observed. A single isolate was resistant to ceftriaxone, however, increased resistance to the other cephalosporins (cefoxitin, ceftiofur, and cephalothin) was observed. Multiple drug resistance (MDR; resistance ≥ 2 antimicrobials) was exhibited in 57% (n=282/499) of the *S. Agona* isolates and 22% (n=111/499) of these *S. Agona* isolates were resistant to 5 or more antimicrobials. A majority of the *S. Agona* isolates originated from cattle (40%; n=202/499) and represented 77% (n=85/111) of the MDR isolates resistant to 5 or more antimicrobials. Cluster analysis indicated that isolates did not group together based on the year isolate was recovered, geographical region, or animal source. However, groupings that were indistinguishable by PFGE appeared to correspond with antimicrobial resistance profiles. These data suggest that *S. Agona* is increasing in prevalence in U.S. cattle present for slaughter and should be monitored further.

24. USDA VetNet and NARMS Working Together. P. J. Fedorka-Cray, C. R. Jackson, A. Douris, C. P. Gresham, M. C. Jackson-Hall, B. M. McGlinchey, M. V. Price.

Abstract: The Bacterial Epidemiology and Antimicrobial Resistance Research Unit (BEARRU) at USDA-ARS houses three programs: the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS), the Collaboration in Animal Health and Food Safety Epidemiology (CAHFSE), and VetNet. The NARMS program conducts susceptibility testing of non-typhoidal *Salmonella*, *Campylobacter*, *E. coli*, and enterococci while the CAHFSE program monitors *Salmonella* from swine on-farm and in plants over time. VetNet provides PFGE analysis of *Salmonella* and *Campylobacter* isolates from both programs. Combined information from NARMS, CAHFSE, and VetNet is used to achieve objectives of the BEARRU project plan.

25. Overview of USDA VetNet. P. J. Fedorka-Cray, C. R. Jackson, A. Douris, C. P. Gresham, M. C. Jackson-Hall, B. M. McGlinchey, and M. V. Price.

Abstract: USDA VetNet commenced in March 2004. The objectives of USDA VetNet are to determine PFGE patterns of *Salmonella* and *Campylobacter* isolates submitted to the National Antimicrobial Resistance Monitoring System (NARMS), to compare USDA VetNet and PulseNet PFGE patterns, and to use the comparative data for surveillance and investigation of foodborne illness outbreaks. In May 2004, the NARMS *Salmonella* database began receiving patterns. By December 2005, 6,673 patterns had been submitted to the database. The top three *Salmonella* serotypes are Newport, Typhimurium, and Kentucky. The most common patterns in the database are Kentucky patterns JGPX01.0003 and JGPX01.0001, and the most common source sites are chicken and bovine. In December 2005, the NARMS *Campylobacter* database began receiving patterns. After one month the database contained 58 patterns all cultured from chicken carcass rinsates. Future objectives for VetNet include making the database available to outside labs for pattern submission and viewing.

26. PulseNet Quality Assurance and Quality Control (QA/QC) Program: An Update. J. Kincaid 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.

27. Above and Beyond "Routine" PFGE Testing. M. Sharp, Gibson, J. A., Kimberly, M. W., Roberts, S. M. and Woron, A. M.

Abstract: Have you ever received a phone call from epidemiology that begins with "Can you do PFGE on...?" In 2005, the Tennessee State Public Health Laboratory in Nashville performed PFGE on 679 routine and 224 non-routine isolates. For this purpose routine testing is defined as isolates of *Salmonella* spp., *Shigella* spp., *E. coli*, *Listeria monocytogenes* and *Campylobacter*. Five of our non-routine testing methods will be presented to demonstrate the importance of sharing successes and failures for the benefit of all. Protocols include *Yersinia enterocolitica*, vancomycin-resistant enterococcus (VRE), *Serratia marcescens*, *Burkholderia cepacia*, and *Staphylococcus aureus*. Methods used were drawn from published literature, the PFGE Protocol Conditions guide and trail and error. All five attempts at non-routine testing provided useful information for the epidemiologists.

2nd Annual National Foodborne Epidemiologists Meeting Abstracts

28. A Large Outbreak of Multi-Drug Resistant *Shigella sonnei* Gastroenteritis

Associated with Daycare Centers — Northwest Missouri, 2005. W. Arvelo*, T. Nguyen, N. Steinmuller, T. Weiser, J. Hinkle, N. Beyer, F. Khan, B. Zhu, E. Mintz and A. Bowen.

*Centers for Disease Control and Prevention, Epidemic Intelligence Service

Background: *Shigella sonnei* causes an estimated 360,000 cases of gastroenteritis annually in the United States. Outbreaks in daycare centers are common and control measures in these settings are complicated by increasing antimicrobial resistance. Between May 1 and October 31 of 2005, >600 persons in the greater Kansas City area were diagnosed with shigellosis. Approximately 82 percent of patients were associated with 63 daycare centers; attendees, employees, and their families were affected. Ninety percent of outbreak strains were resistant to ampicillin and trimethoprim-sulfamethoxazole, drugs commonly used for treatment. A case-control investigation was conducted among licensed daycare centers (LDCs) to determine transmission risk factors.

Methods: Thirty-nine LDCs in the greater Kansas City area with confirmed shigellosis cases during the outbreak period were included in the case-control investigation. Case LDCs had a secondary attack rate $\geq 2\%$ and control LDCs had a secondary attack rate $< 2\%$. At each LDC, the director and a staff member were interviewed regarding knowledge and practices concerning shigellosis and in regards to hygiene behavior, such as handwashing and diapering; an observational checklist was also performed.

Results: Eighteen case- and 21 control-LDCs were enrolled. No significant differences in knowledge and practices about handwashing or diapering were found. LDCs with ≥ 1 sink in every room (Odds Ratio [OR] 0.11; Confidence Interval [CI] 0.02 – 0.52) or a diapering station in every room with diapered children (OR 0.10; CI 0.02 – 0.62) were less likely to be case-LDCs.

Conclusions: Hand hygiene can prevent the transmission of shigellosis and could reduce the use of antimicrobials. However, hygiene knowledge is not sufficient. Appropriate handwashing and diapering infrastructure is also necessary to minimize spread of shigellosis within daycare centers and to the community.

Key words: *Shigella sonnei* infections, diarrhea, multi-drug resistance, daycare centers, epidemiology, disease outbreaks

29. Novel Strategy for Detecting Staphylococcal Enterotoxin During a Foodborne

Outbreak. M. C. Bañez Ocfemia*, A. Huang, J. Besser, E. Cebelinski, S. Jawahir, K. Gall, E. Shade, C. Braymen, L. Burnsed and D. C. Hunt.

* Kansas Department of Health and Environment, Bureau of Epidemiology and Disease Prevention

Background: Staphylococcal food poisoning, caused by enterotoxins produced by *Staphylococcus aureus*, is a common public health problem. Methods for directly detecting enterotoxins in human stool samples are not widely available. We describe a polymerase chain reaction (PCR) method for detecting staphylococcal enterotoxin

A (SEA) directly from stool. This method was used in a foodborne outbreak investigation that occurred in Kansas in December 2005.

Methods: We conducted a retrospective cohort study. A case was defined as a person with vomiting or diarrhea (three or more loose stools within 24 hours) who had eaten the suspected meal within the previous 24 hours. Six patients submitted stool for bacterial culture and parasitology. An environmental investigation was conducted. Four foods that were prepared for the meal, but not served, were collected for microbiological testing.

Results: Of responders who had eaten the suspected meal, 138/306 (45%) met the case definition. Median incubation time was 3.3 hours. The most common symptoms were nausea (92%), stomachache (91%), diarrhea (87%), and vomiting (80%). Sausage was implicated epidemiologically (RR: 3.24; 95% confidence interval = 1.30–8.07). Environmental investigation revealed that food temperatures were not monitored during preparation, storage, or before serving. SEA-producing *S. aureus* were isolated from all six stool samples. All isolates were indistinguishable by pulsed-field gel electrophoresis. SEA DNA was identified by PCR in four of six preserved stool samples. No *S. aureus* or enterotoxin was identified in the food samples.

Conclusions: Epidemiologically, this outbreak is consistent with staphylococcal foodborne illness. Culture revealed the presence of *S. aureus* in stool; however, samples were suboptimal, and the organism could not be quantified. PCR testing supported the hypothesis that this outbreak was caused by SEA-producing *S. aureus*. Further evaluation, including testing of control stools, is warranted to determine the utility of the PCR in the outbreak setting.

30. Saxitoxin and Pufferfish, Case Study of a Florida Outbreak, 2002 through 2004.

D. Bodager* and R. Hammond.

*Florida Department of Health, Bureau of Community Environmental Health

Objectives:

1. Participants will be able to describe the prevalence of saxitoxin in humans
2. Participants will learn of new source of saxitoxin poisoning in humans
3. Participants will understand the methods of intervention in the transmission of saxitoxin to humans

While paralytic shellfish poisoning outbreaks have been reported throughout the world, they have not previously been reported in Florida. Human saxitoxin poisonings called paralytic shellfish poisonings have been associated with the consumption of clams, mussels, cockles, oysters, and scallops, which contain 200-1000 µg saxitoxin/100g. Nonfilter feeders such as fish, lobster, crabs and shrimp harvested from the same waters have been generally considered safe to eat.

Saxitoxins are produced by dinoflagellates of the Gonyaulacoid family and by some freshwater cyanobacteria. Cooking or freezing cannot destroy saxitoxin and the toxin does not change the taste or odor of food.

Florida documented 23 cases of saxitoxin poisoning during 2002 and 2003. Saxitoxin was present in five case urine samples ranging from 2.2 to 106 ng/ml. Southern pufferfish (*Sphoeroides nephelus*) collected from the Indian River contained saxitoxin concentrations ranging from 200-5300 µg saxitoxin equivalent toxicity/100g. Lower

concentrations of the toxin were found in samples from the South Banana River. The regulatory limit for saxitoxin in shellfish is 80 µg saxitoxin equivalent/100g. The samples were very toxic in the body meat, skin, mucous and gut while the liver had relatively low concentrations of the toxin. A variety of finfish and shellfish species, including hard clams, were tested for toxins and found to be negative for the presence of saxitoxin. These samples were negative for tetrodotoxin. Successful risk communication was a critical element in attempting to prevent additional illnesses of saxitoxin poisoning from consumption of pufferfish.

31. Restaurant-Associated Outbreak of *Salmonella* Heidelberg Infections. C.

Burnett*, M. Tobin-D'Angelo, C. Shuler, Sandra Toye, Robert Finton, J. Benson and R. Manning.

*Georgia Department of Human Resources, Division of Public Health

Background: On December 9, 2005, through routine interviews of reported Salmonellosis cases, the Georgia Division of Public Health (GDPH) was notified of several cases of *Salmonella* Heidelberg among patrons of a Fulton County, Georgia restaurant. The cases had all eaten at the restaurant on November 30. An investigation was begun to determine the scope of the outbreak and identify the source of infection.

Methods: GDPH staff contacted the restaurant and found that restaurant staff were aware of additional cases. The restaurant manager provided contact information for these cases as well as a list of the names and phone numbers of all persons who had made reservations at Restaurant A on November 30. The restaurant also provided a copy of the bill for each group, showing all the menu items ordered by group members. GDPH interviewers attempted to contact everyone who made reservations the night in question. Names and contact information for other members of each dining party were obtained from the primary contacts. A cohort study was conducted by interviewing as many diners as possible with a standardized questionnaire. Ill patrons were encouraged to provide stool specimens. *Salmonella* isolates were serotyped and PFGE analysis was conducted by the Georgia Public Health Laboratory. Additional case finding was conducted by contacting persons with *Salmonella*-positive stool cultures to determine if they had eaten at Restaurant A. Food and environmental samples, and stool specimens from all food handlers were collected and tested for *Salmonella*.

Results: A total of 85 restaurant patrons who ate at the restaurant on November 30, 2005 were interviewed. Of these, 33 (39%) reported experiencing a diarrheal illness within 5 days of eating at the restaurant. Eight patients had positive stool cultures for *Salmonella* Heidelberg. PFGE testing of isolates revealed indistinguishable patterns among all 8 isolates. Illness was found to be statistically associated with consuming foods containing hollandaise sauce made with pooled, raw shell eggs. The environmental investigation found potential problems with preparation and holding temperatures. No food handlers reported gastrointestinal illness during the period before or after the outbreak, and all food-handler stool cultures were negative for *Salmonella*.

Conclusions: At least 33 persons became infected with *S. Heidelberg* after consuming hollandaise sauce containing raw shell eggs at Restaurant A. Pooling of

raw shell eggs, inadequate heating and holding temperatures all contributed to this outbreak. Like *S. Enteritidis*, *S. Heidelberg* may contaminate eggs through the trans-ovarian route. Although pooling of raw shell eggs in restaurants is strongly discouraged by the U.S. Food and Drug Administration, this practice still occurs. After the outbreak was identified, Restaurant A began using only pasteurized eggs in their sauces. No additional cases of *S. Heidelberg* associated with Restaurant A have been identified.

32. Epidemiology of Foodborne Outbreaks of Undetermined Etiology, FoodNet

2001-2004. C. J. Snider*, D. J. Vugia, A. Cronquist, Q. Phan, C. Burnett, L. Edwards, E. Swanson, D. L. Morse, W. E. Keene, K. E. Fullerton, M. Lynch, T. F. Jones and the EIP FoodNet Working Group.

*Centers for Disease Control and Prevention, Foodborne and Diarrheal Diseases Branch
Background: Annually, an estimated 62 million cases of acute foodborne gastroenteritis in the US are due to undetermined etiologies. Outbreak investigations serve an important role in understanding the epidemiology of foodborne pathogens.
Methods: We analyzed data from CDC's national electronic Foodborne Outbreak Reporting System (eFORS) with supplemental outbreak forms completed by Foodborne Diseases Active Surveillance Network (FoodNet) sites since 2001 to assess barriers to outbreak investigations. CDC criteria were used to define etiologies.
Results: A total of 943 outbreaks were reported via eFORS in FoodNet sites; 888 (94%) were linked to supplemental forms. Of these, 398 (45%) outbreaks had an undetermined etiology. Among outbreaks of undetermined etiology, only 112 (28%) outbreaks had stool specimens collected from ≥ 2 persons. Among outbreaks with stools collected from ≥ 2 persons, 62 (55%) outbreaks had an investigation begun within one week of outbreak onset, and stool samples collected within one week of onset of first patient illness. Overall, only 16% of outbreaks of undetermined etiology appeared to have an investigation begin and adequate number of stools tested within one week of illness onset.

Conclusion: In 84% of foodborne disease outbreaks of undetermined etiology a thorough investigation was not performed promptly, most commonly because 72% of these outbreaks had inadequate specimen collection. Physicians play a vital role in an outbreak investigation by promptly collecting specimens and reporting suspected outbreaks to health departments. These are important steps to ensure a successful investigation.

33. *Salmonella Paratyphi A* in the United States: travel and quinolone resistance. S.

Gupta*, J. Whichard, M. Omondi, F. Medalla, T. Chiller and E. Mintz.

*Centers for Disease Control and Prevention, Epidemic Intelligence Service
Abstract: Little is known about the epidemiology of paratyphoid fever in the United States. Quinolone antibiotics are often used to treat paratyphoid fever, which is most often caused by *S. Paratyphi A*. Resistance to the quinolone nalidixic acid correlates with reduced susceptibility to the fluoroquinolone ciprofloxacin, and treatment failures with ciprofloxacin have been reported in patients with nalidixic acid-resistant *Salmonella*.

For one year beginning April 1, 2005, state and local health departments agreed to send all *S. Paratyphi A* isolates to CDC for susceptibility testing and interview the patients with a standard questionnaire.

Through October 15, 64 *S. Paratyphi A* cases were reported, 35 were interviewed and 16 isolates were tested. Of those interviewed, median age was 31 years (range, 5–67); 54% were male. Most (91%) isolates were from blood. 34 (97%) patients reported fever (duration: range, 2–30 days, median 7 days) and 17 (49%) had diarrhea; 24 (69%) were hospitalized. No deaths or outbreaks were reported. 33 (94%) patients traveled internationally ≤ 30 days before illness onset. Of these, 28 (85%) traveled to South Asia. All 15 adults with available treatment information received fluoroquinolones, compared to 4 (57%) of 7 children. 12 (75%) of 16 isolates tested were nalidixic acid resistant. Of nine cases with resistance and travel information available, 5 (83%) of 6 nalidixic acid resistant isolates were from travelers to South Asia, compared to 1 (33%) of 3 susceptible isolates ($p=ns$).

Most cases of *S. Paratyphi A* in the United States are associated with travel, especially to South Asia. Unlike typhoid fever, however, no vaccine to protect travelers from paratyphoid fever is available. Quinolone resistance is frequent with *S. Paratyphi A* and may be more common among travelers to South Asia. Treatment decisions should take these findings into consideration.

Keywords: Paratyphoid fever, antimicrobial drug resistance, travel.

34. Statewide Cyclospora Outbreak, Florida 2005. R. Hammond* and K. Ward.

*Florida Department of Health, Bureau of Community Environmental Health

Objectives:

- 1) Participants will be able to discuss the etiology of *Cyclospora cayetenensis*.
- 2) Participants will be able to name typical vehicles of cyclosporiasis.
- 3) Participants will be able to discuss implications for the occurrence of possible future outbreaks from this parasite.

This outbreak was caused by *Cyclospora cayetanensis*. In mid-April, 2005, a private laboratory reported a dozen cases of cyclosporiasis to the Florida Department of Health. The total number of cases reported in 2004 was 9, and the average for 2003-2005 for reporting week 14, ending April 16 (the week the positive results were received from the private lab) was 1.67, 20% higher than normally expected. By reporting week 17, the percent increase was 162%, a clear indication of a possible outbreak. Cases were reported from numerous counties with no initial apparent pattern. This began the investigation into the largest reported cyclospora outbreak in Florida history with 592 cases. The investigation was a multi-county, multi-agency, and state-federal collaborative effort requiring intensive coordination. Private and state laboratories played an integral part in case-finding as did the efforts of several county health departments who investigated clusters of illness related to exposure at specific restaurants as well as sporadic cases. Through investigation clusters in three primary counties, a food product was identified, enabling a formal traceback by the U.S. Food and Drug Administration.

Typical vehicles of past *Cyclospora* outbreaks include raspberries, basil, lettuce, snow peas and water. Though water has been implicated, 90% of outbreaks of Cyclosporiasis are foodborne. Cyclosporiasis is endemic in many developing

countries and is often associated with diarrhea in travelers to Asia, the Caribbean, Mexico and Peru. CDC reports that there have been 5,000 cases reported in the last 5 years. Illness is caused by *Cyclospora cayetanensis*, a single celled protozoan with symptoms of watery diarrhea, nausea, loss of appetite, abdominal pain, fatigue and weight loss. The case fatality rate is very low. The incubation period is 1-7 days, usually about 1 week and the ensuing illness can last anywhere from 1-3 weeks.

35. Foodborne Hepatitis A in Florida, 1994-2005. R. Hammond.*

*Florida Department of Health, Bureau of Community Environmental Health

Objectives:

- 1) Participants will understand the relative risk of foodborne Hepatitis A in Florida versus other foodborne illnesses.
- 2) Participants will be able to discuss the incidence of foodborne Hepatitis A in foodworkers in Florida.
- 3) Participants will be able to name common vehicles for foodborne Hepatitis A in Florida.

Hepatitis A is endemic in many underdeveloped countries where children often are asymptomatic or have only mild illness. Onset of illness can be abrupt with symptoms of fever, malaise, anorexia, nausea and abdominal discomfort followed in a few days by jaundice. Hepatitis A has a relatively low case fatality rate, although 2 cases from Florida foodborne outbreaks are known to have died from fulminant hepatitis. Because of the lengthy incubation period of 15 to 50 days, foodborne hepatitis A outbreaks can be difficult to investigate. Transmission is person to person by the fecal-oral route, prompting handwashing campaigns to prevent the spread of illness. During foodborne hepatitis A outbreaks, IG should be administered to other food workers if a food worker is the index case. IG of patrons is considered using the following criteria:

- a) Were infected food workers involved in the preparation of ready-to-eat foods?
- b) Were deficiencies noted in the infected food workers' hygiene, or did the food worker have diarrhea/
- c) Can IG be given within 2 weeks of the last exposure?

While several hepatitis A outbreaks have garnered much attention in the media, ten year's of foodborne disease data show that hepatitis A is not a primary cause of foodborne illness in Florida. This poster will discuss the prevalence of foodborne hepatitis A in Florida, hepatitis A in Florida foodworkers, the relative risk of foodborne hepatitis A versus other causes of the disease, and educational efforts and barriers to prevent the spread of hepatitis A.

36. Salmonella Outbreaks in Restaurants in Minnesota, 1995-2003: Evaluation of the Role of Infected Foodworkers. C. Medus*, K. Smith, J. Bender, J. Besser and C. Hedberg

* Minnesota Department of Health, Acute Disease Investigation and Control Section

Abstract: Restaurant-associated salmonellosis outbreaks in Minnesota during 1995-2003 (n=23) were reviewed to characterize the role of infected foodworkers. The median duration of the outbreaks was 21 days (range, 1-517). The median number of culture-confirmed patron-cases per outbreak was seven (range, 1-36). The median

incubation for patron-cases ranged from 9 hours-5.9 days. A specific food vehicle was implicated in four outbreaks, and suspected in five. *Salmonella* of the same serotype and pulsed-field gel electrophoresis subtype as patrons was recovered from foodworkers in 19 outbreaks. Overall, 12% (129/1,033) of foodworkers tested positive for *Salmonella*. Sixty-four of 121 (53%) positive foodworkers reported not having recent gastrointestinal illness. Overall, the median duration of shedding was 16 days. Among foodworkers who reported gastrointestinal illness, the median shedding duration was 30 days, vs. 3 days for asymptomatic foodworkers. Positive environmental samples were recovered in four of 12 (33%) outbreaks. A specific food vehicle was not identified in any outbreaks with positive environmental samples. The median duration of outbreaks with positive environmental samples (187 days) was significantly longer than the median duration of outbreaks with negative environmental results (26 days, $p=0.03$). A higher proportion of positive foodworkers (22% vs. 8%) was identified in outbreaks with positive environmental samples. *Salmonella* outbreaks in restaurants are frequently prolonged, yet have a small number of confirmed patron-cases. Prolonged outbreak durations suggest a persistent reservoir of contamination. Infected foodworkers likely serve as an important source for transmission; therefore, assessment of foodworker infection is essential for controlling restaurant outbreaks.

37. Delayed Stool Collection and Norovirus PCR Results. M. S. Plantenga*, P. R.

Cieslak, W. E. Keene, C. Biggs, J. Terry and L. Grenz.

*Oregon Department of Human Services

Background: Perhaps because of a real increase in disease or simply from better reporting, Oregon has in the past few years witnessed an explosion in reported outbreaks of gastroenteritis with clinical features characteristic of Norwalk-like virus (NLV, norovirus) infection. Given the short duration of symptoms (typically <48 h) in NLV infection, it is not always feasible to obtain specimens from symptomatic individuals. We evaluated the importance of early specimen collection in confirming etiology.

Methods: Oregon local health department staff, collected fecal specimens from likely outbreaks and tested for NLV by reverse transcriptase polymerase chain reaction (RT-PCR), using norovirus consensus primers. PCR results were linked to epidemiological information including dates of exposure, diarrhea onset, diarrhea cessation, and specimen collection. **Results:** We investigated 103 outbreaks during July 1, 1999 — August 31, 2003, where-in NLV was confirmed by RT-PCR in stools from at least two cases. These included foodborne and person-to-person outbreaks. During these investigations we collected and tested one specimen from each of 543 persons; 421 (78%) were positive. Diarrhea onset and stool collection dates were available for 346 individuals (64%), of whom 283 (82%) were positive. Table 1 shows the likelihood of a positive test by time since onset. Dates of diarrhea cessation and specimen collection were available for 149 (27%) of 543 individuals, of whom 128 (86%) were positive. Table 2 shows the likelihood of specimens testing positive by time since resolution of diarrhea.

Conclusions: PCR-positivity for NLV does not begin to decline until 1 week after onset and—while somewhat more variable—for almost that long after symptoms resolve. NLV outbreaks can be readily confirmed if specimens are collected at any time in the first week after onset and quite possibly even through the 2nd week. We note that we cannot assess infectivity from these data.

38. Utah’s Enteric Disease Outbreak Investigation Model. M. Poulson* writing for Utah’s Enteric Disease Investigation and Management Workgroup.

*Utah Department of Health

Abstract: A working group of epidemiologists, laboratorians, public health nurses, and environmental health staff from state and local public health agencies in Utah has developed written protocols for the investigation of enteric disease outbreaks, titled “Enteric Disease Outbreak Investigation Model”. The Model is directed toward local investigative teams and allows for different levels of experience. With an innovative approach to outbreak investigation, the Model consists of three investigative stages with clear evaluation steps, encouraging investigators to discuss as a group at what point the investigation should be finalized. Suggestions and recommendations contained in the Model are not required to be completed, rather presented as guidelines or best practices. The working group is taking on the challenge of marketing the use of the Model by presenting it at professional environmental and public health conferences; making hard copies available to local health departments for quick reference; and encouraging local health departments to institute team orientation and training sessions that include individuals with epidemiologic, environmental, nursing, legal, and public information expertise.

39. An Outbreak of Enterotoxigenic *Escherichia coli* After an International Event. R. Reporter*, A. Gallagher, J. Sturgeon, M. Bird, C. Crandall and L. Mascola.

*Los Angeles Department of Health Services

Background: In October, 2004, the Los Angeles County Department of Health Services (LACDHS) investigated a large outbreak of enterotoxigenic *Escherichia coli* (EPEC) infections following an international religious event. From 1975-2003, only 26 outbreaks of EPEC have been reported in the US.

Methods: A case was defined as a person who ate food at the event and reported diarrhea (*2 loose stools/24 hrs) within 6-72 hours after eating. Non-ill attendees were selected as controls. Interviews were conducted using a standardized questionnaire. Stool samples were tested in the LACDHS Public Health Laboratory (PHL) for bacterial, parasitic and viral pathogens and *E. coli* isolates were subtyped by PFGE. Serotyping and PCR toxin testing for heat labile (LT) and heat stable (ST) enterotoxins were performed by CDC and CA Dept. Health Services.

Results: About 600 persons attended the event from 8 states and 4 other countries?. Of 76 randomly obtained interviews, 56 were cases; 20 were controls. Symptoms were: non-bloody diarrhea (98.2%), abdominal cramps, (85.7%), nausea (51.8%), fatigue (48.2%), headache (44.6%), bodyaches (30.4%), fever (26.8%) and vomiting (19.2%). Average duration was 5.2 days (range <1-11 D). Incubation ranged from 10-67 hours. Medical care was sought by 6 (10.7%). Analysis indicated the strongest

association with eating any beef dish (OR=12.27, p<.0001). The beef was prepared the night before and may have been held at room temperature on the day of the event. Laboratory analysis revealed 6/9 stool specimens were positive for enterotoxigenic *E. coli*, serotype O169:non-motile, ST positive. PFGE of 5/6 isolates yielded 2 indistinguishable patterns and 3 isolates with patterns differing by a total of 1-3 bands by *Xba*I and *Bln*I. Frozen leftover vegetables were negative for *E. coli*.

Conclusion: An outbreak of ETEC was likely caused by improperly stored beef. Restaurants should follow recommended food handling practices. Duration of symptoms, longer incubation and low vomit/diarrhea ratio helped LACDHS hypothesize the outbreak etiology was ETEC. ETEC is not detected by routine stool culture. If ETEC is a suspected cause of an outbreak, specific testing must be requested from the PHL with the stool specimens.

40. Outbreaks Caused by Drug-Resistant versus Pan-Susceptible Non-Typhi

Salmonella: NARMS, 1996-2004. L. M. Stancik*, T. J. Barrett, C. R. Braden and T. M. Chiller.

*Centers for Disease Control and Prevention, Foodborne and Diarrheal Diseases Branch

Background: There is increasing evidence that infections caused by non-Typhi *Salmonella* that are resistant to antibiotics have more severe health consequences than pan-susceptible infections. We examined the clinical characteristics of outbreaks caused by resistant and pan-susceptible *Salmonella*.

Methods: All isolates of non-Typhi *Salmonella* received by CDC from outbreaks between 1996 and 2005 were tested by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS: EB) lab. In this analysis, outbreaks were included if susceptibility data were available for ≥ 3 isolates. Outbreaks were classified as “resistant” or “pan-susceptible” using the following algorithm. If an isolate was resistant to ≥ 1 agent then the isolate was “resistant.” If all or most of the isolates in an outbreak were resistant then the outbreak was classified as “resistant,” all others were “pan-susceptible.” Resistant outbreaks were classified as “R-Type AC/KSSuT” when isolates were at least resistant to ampicillin, chloramphenicol or kanamycin, streptomycin, sulfamethoxazole, and tetracycline.

Results: From 1996-2005, CDC tested ≥ 3 isolates from 80 outbreaks of non-Typhi *Salmonella*. Of these outbreaks, 25 (31%) were classified as resistant and 55 (69%) as pan-susceptible. The three serotypes *S. Typhimurium*, *S. Newport*, and *S. Enteritidis* made up 80% of the outbreak isolates. Of the 25 resistant outbreaks, 24 (96%) were at least R-Type AC/KSSuT. Hospitalization occurred in 14% (51/353) of infected persons in 10 outbreaks all caused by R-Type AC/KSSuT strains. In contrast, hospitalization occurred in only 6% (132/2,238) of infected persons in 17 outbreaks caused by pan-susceptible strains. Mortality among resistant and pan-susceptible outbreaks did not differ. Of the 80 outbreaks, 40 (50%) had identified sources: 6 were meat and 5/6 (83%) of these were resistant outbreaks.

Conclusions: Outbreaks caused by resistant *Salmonella* were found to have more days of hospitalization than those caused by pan-susceptible strains. This supports growing evidence that resistant *Salmonella* infections are associated with a worse clinical course.

41. City of Houston’s Food Safety Progress Review Toward Healthy People 2010 Goals. B. J. Thorne*, M. P. Linn, S. Long, and Y. Zhang.

*Houston Department of Health and Human Services

Objectives:

1. To present a summation of progress achieved in meeting the targets of selected objectives in the Food Safety focus area of “Healthy People 2010.”
2. To present the laboratory confirmed incidence rates of key food borne pathogens that were reported to the City of Houston, Bureau of Epidemiology in 1997 and in 2004 and tracked by “Healthy People 2010”.
3. To present key challenges and current strategies utilized at the local level.

Abstract:

Every 5 years, the Bureau of Epidemiology in the Houston Department of Health and Human Services (HDHHS) does a comprehensive review of the epidemiology of reportable diseases in the City of Houston. Laboratory incidence rates of key food borne pathogens in 2004 and for the 5-year period (2000-2004) will be charted alongside selected objectives in the Food Safety area in the Healthy People 2010 goals. Hence, progress in achieving those goals to date will be tracked. Current key challenges and strategies practiced at the local level will be highlighted.

42. Salmonella Typhimurium Outbreak Associated with Cake Batter Ice Cream from a Nationwide Ice Cream Chain. S. D. Wedel*, W. E. Keene, K. Macdonald, T. Powell, E. Salehi, H. Kassenborg, D. J. Boxrud, G. Zhang, C. R. Braden, M. Lynch and K. Smith.

*Minnesota Department of Health

Background: On June 29, 2005, the Minnesota Department of Health identified four *Salmonella* Typhimurium isolates with a pulsed-field gel electrophoresis (PFGE) subtype that was new to the PulseNet national database. Four patients reported eating cake batter flavor ice cream from two separate outlets of national ice cream chain A.

Methods: The PulseNet national database was queried to identify potential cases in other states. A case was defined as infection with an *S. Typhimurium* isolate that matched the outbreak PFGE pattern, and illness onset since May 2005. All cases were interviewed with a standard questionnaire. State and federal officials conducted a traceback of ice cream ingredients.

Results: We identified 25 cases in nine states (MN, 5; OR, 5; WA, 5; VA, 3; OH, 2; CA, IL, MA, MI, PA, 1 each); 24 reported eating cake batter ice cream from Chain A. The median age of cases was 13 years (range, 2–32 years). The median incubation was 4 days (range, 1–7 days). Illness onset dates ranged from May 21 to July 4; four cases were hospitalized. Chain A voluntarily recalled cake batter ice cream on July 1. This flavor’s ingredients included a pasteurized liquid sweet cream base and a commercial powdered cake mix. The sweet cream base was used in numerous other ice cream flavors, but the cake mix was used only in cake batter ice cream. The cake mix comprised spray-dried egg whites, flour, and several low-risk components. Tracebacks in Minnesota, Oregon, and Virginia implicated a single lot of cake mix produced on April 14, 2005. No manufacturing anomalies were identified for this lot; but two cake mix samples yielded the outbreak strain of *S. Typhimurium*. The Food

and Drug Administration warned food retailers that cake mixes and flour are not considered “ready to eat” and should be heat processed before consumption.

Conclusion: The vehicle for this outbreak was ice cream made with a contaminated cake mix ingredient. While the ultimate source of contamination was not confirmed, we recommend a review of the efficacy of spray-drying egg whites as a kill step for *Salmonella*. Routine and rapid subtyping of bacterial isolates, coupled with a vigorous epidemiological response, is critical to identifying and abating multi-state outbreaks.