REPORT OF THE COMMITTEE ON SALMONELLA

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Vice Chair: Dr. Patrick L. McDonough, Ithaca, NY

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The Committee met from 12:30 pm-6:10 pm October 24, 2004, with 63 members and guests in attendance. Chair Dr. David M. Castellan presided assisted by Vice Chair Dr. Patrick L. McDonough. Two subcommittees were appointed at the end of the meeting - one to study and to write comments for the newly proposed Food and Drug Administration (FDA) *Salmonella enteritidis* program, and the second to monitor Salmonella Performance Standards during the coming year. Two resolutions were proposed at the end of the meeting.

Dr. Andy Rhorer, United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), Senior Coordinator**,** presented information on the National Poultry Improvement Plan (NPIP) for the calendar year 2003. He also gave a Pullorum case report. In 2003, there were eight isolations/outbreaks of *Salmonella pullorum* (both standard and intermediate types) reported to NPIP. There were 42 isolations/outbreaks from 27 flocks of *Salmonella pullorum* reported from January 1 to October 1, 2004. There have been no isolations of *Salmonella gallinarum* since 1988 in any type poultry.

Dr. Kathy Ferris, USDA-APHIS-VS National Veterinary Services Laboratories (NVSL), Ames, Iowa, presented the NVSL Salmonella serotyping report for 11,493 animal, avian and epidemiologically related sources for the time period July 1, 2003 – June 30, 2004. The most frequently identified serotypes were *Salmonella typhimurium, S. newport, S. heidelberg, S. kentucky,* and *S. senftenberg*.

The *Salmonella* are isolated from cases of clinical disease and from herd and flock monitoring and are submitted by animal disease diagnostic laboratories throughout the USA. Data are included on Salmonella isolated by the Food Safety and Inspection Service (FSIS) as a result of HAACP (Hazard Analysis and Critical Control Point) testing. Data generated from the serotyping of research isolates are not included in this report.

A total of 228 serotypes were identified from isolates recovered from animals, their environment, or feed in 37 states and the District of Columbia. The 10 most common serotypes accounted for 66% of the total isolates reported. *Salmonella typhimurium, S. newport, S. derby, S. heidelberg, S. kentucky, S. senftenberg,* and *S. muenster* are among the 10 most common serotypes from both monitor and clinical cases.

Although the total number of *S. typhimurium* isolates is less, the percent of total isolates identified as *S. typhimurium* increased from 15% in 2003 to 20% this year. Twenty-five percent of clinical isolates were *S. typhimurium* compared to 21% last year, and 15% of monitor isolates were *S. typhimurium. S. typhimurium* is one of the most common serotypes isolated from cattle, chickens, swine, and horses again this year, but there were only 20 isolates of turkey origin identified as *S. typhimurium*. Of the total isolates identified as *S. typhimurium*, 58% were *S. typhimurium* var. *copenhagen* and 42% were *S. typhimurium*.

Again this year, 8% of all isolates were identified as *S. newport*. It was the second most common serotype for the first time, and was the most common serotype isolated from cattle in cases of clinical disease and the second most common from horses with clinical disease. The majority of *S. newport* (66%) was of cattle origin and 18 % were from horses, compared to 72% and 14% last year.

There were 32 isolates identified this year as *S. pullorum* (standard

strain), isolated from chickens in nine states. It was the most common serotype, along with *S. heidelberg*, identified from cases of clinical disease in chickens. Last year there were 18 *S. pullorum* (standard) and 2 *S. pullorum* (intermediate) identified from cases of clinical disease in chickens, none were reported the previous year, and 4 *S. pullorum* (standard) were reported in 2000-2001. *S. pullorum* (standard) was isolated from the intestinal tract of a rat trapped on a farm where *S. pullorum* had been identified. It was also isolated from chickens and ducks on a neighboring farm. These isolates exhibited identical pulsed-field gel electrophoresis (PFGE) patterns.

Dr. James McKean, Iowa State University and Dr. Elizabeth Wagstrom, National Pork Board, gave a report on the initiatives to minimize *Salmonella* in swine and pork products. *Salmonella* prevalence on market hog pork carcasses, as measured by FSIS performance standard testing, has declined from a baseline of 8.7% to 3.2% for 2003 samples. Large slaughter establishments had a *Salmonella* positive rate in market hogs at 2.5%. This low rate at plants is also reflected in retail meats.

Although the level on carcasses and at retail is low, *Salmonella* tops the list of pre-harvest research priorities for the National Pork Board. Among these research priorities are a risk assessment for *Salmonella* throughout the pork production chain, and also research into the effect of pre-harvest interventions on final product contamination.

Interest in pre-harvest *Salmonella* control was spurred by the establishment of the Danish *Salmonella* Control program in 1995. Similar programs have been adopted recently in other European Union countries. The National Pork Board will be taking a study trip to Denmark in January, 2005 to fully investigate the benefits (public health and animal health) and costs of the program. Additionally, the National Pork Board is hosting a *Salmonella* Colloquium among members of the entire pork chain to discuss a U.S. program.

Additional National Pork Board efforts in *Salmonella* control include the development of critical literature reviews of pre-harvest interventions. The Board's *Salmonella* Technical Working Group is also working with two American Association of Veterinary Laboratory Diagnosticians (AAVLD) committees to develop minimum standards for *Salmonella* isolation and determination of prevalence within a herd.

Dr. Paula Fedorka-Cray, USDA, Agricultural Research Service (ARS), provided the Committee with an update of the USDA multi-agency-program-the National Antimicrobial Resistance Monitoring System for enteric bacteria (NARMS).

Recognizing the potential utility of antimicrobial susceptibility testing for monitoring trends in antimicrobial resistance development and because of the public health concerns associated with the use of antimicrobials in livestock, the program was proposed by the Food and

Drug Administration (FDA), Center for Veterinary Medicine (CVM). This program was developed particularly as a post-marketing activity to help ensure the continued safety and efficacy of veterinary antimicrobials, especially fluoroquinolones.

In 1996, the FDA, USDA, and Centers for Disease Control and Prevention (CDC) initiated NARMS to prospectively monitor changes in antimicrobial susceptibilities of zoonotic pathogens from human and animal diagnostic specimens, from healthy farm animals, and from raw product collected from federally inspected slaughter and processing plants. Non-typhoid *Salmonella* was selected as the sentinel organism. Additional organisms were added to the program, and NARMS currently monitors antimicrobial susceptibility in non-typhoid *Salmonella*, *Escherichia coli*, *Campylobacter* and *Enterococcus* in humans and animals. *Salmonella typhi*, and *Listeria, Vibrio* and *Shigella* isolates collected from humans are also tested and the program has also expanded to include testing of isolates from retail meat.

The animal arm of NARMS resides at the USDA-ARS laboratory in Athens, GA, the human arm resides at CDC in Atlanta, GA, and the retail arm resides at FDA Office of Regulatory Affairs (ORA) in Laurel, MD.

The goals and objectives of the monitoring program are to 1) provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms from the human and animal populations; 2) facilitate the identification of resistance in humans and animals as it arises; 3) provide timely information to veterinarians and physicians; 4) prolong the life span of approved drugs by promoting the prudent and judicious use of antimicrobials; and 5) identify areas for more detailed investigation. Program information may be accessed at www.fda.gov/cvm/index/narms/narms_pg.htm. Additional information on results from the animal isolate testing, including percent resistance by animal species for each year testing has been conducted, can be found at www.arru.saa.ars.usda.gov. Dr. Fedorka-Cray distributed a CD-ROM containing a summary of the NARMS project data for the time period 1997 to 2003.

Dr. Elizabeth A. Krushinskie, U. S. Poultry & Egg Association in Georgia, gave the Broiler Industry perspective of the *Salmonella* Performance Standards.

Foodborne illness caused by *Salmonella* contamination of raw meat and poultry is estimated by the USDA Economic Research Service to result in 1.4 million cases at a cost $3 billion annually. Because of this human disease burden, FSIS issued the *Pathogen Reduction: Hazard Analysis and Critical Control Point (PR/HACCP) Systems* final rule in 1996 and the *Salmonella* Performance Standards in 1998. These regulations required raw meat and poultry processing plants to meet specific *Salmonella* Performance Standard (SPS) goals for a variety of

classes of raw products, including broilers and ground chicken, in order to verify that industry PR/HACCP systems are effective in controlling disease-causing bacteria with the goal of reducing human Salmonellosis cases.

The poultry industry, in turn, has implemented a large variety of new and innovative *Salmonella* reduction strategies both in the plant and pre-harvest. These include implementation of HACCP programs, improved process control, use of antimicrobial sprays and rinses in processing, and optimization of pH and chlorine levels in the chiller. They also developed and implemented numerous food safety best management practices during production and innovative vaccination strategies aimed at reducing *Salmonella* carriage into the processing plant.

Earlier this year, FSIS issued a progress report on the *Salmonella* Performance Standard testing results from 1998-2003. The results reported for broilers showed that the *Salmonella* prevalence from all sizes of establishments for all six years of testing was consistently lower than the prevalence reported from agency baseline studies and surveys conducted before PR/HACCP implementation. The *Salmonella* prevalence increased slightly from 11.5% in 2002 to 12.8% in 2003, however, the 2003 overall level for broilers was still well below the baseline prevalence of 20.0%. The results of six years of testing also showed that the approximately 90% of completed "A" sets achieved the performance standard requirements for broilers from all sizes of establishments. The percent of sample sets meeting the SPS declined slightly from 88.2% in 2002 to 86.6% in 2003.

Overall, the broiler industry is achieving the level mandated by the *Salmonella* Performance Standard by a wide margin (12.8% vs. 20%), but the prevalence rates achieved have been essentially static for the past six years showing no trend toward reduction. Interestingly, the rate of human *Salmonella typhimurium* cases has declined precipitously in spite of the stasis in the broiler SPS results indicating that other factors may have been more influential in reducing the incidence of Salmonellosis through this time period. Further investigation is needed to elucidate these relationships.

Dr. John Dunn, CDC, Foodborne & Diarrheal Diseases Branch, Epidemic Intelligence Service, gave a report on *Salmonella* trends in Emerging Infections Program Foodborne Diseases Active Surveillance Network (FoodNet). In the United States, an estimated 76 million persons contract foodborne and other acute diarrheal illnesses each year. FoodNet collects data on diseases caused by enteric pathogens transmitted commonly through food. FoodNet quantifies and monitors the incidence of these infections by conducting active surveillance for laboratory-diagnosed illness. During 1996-2003, the FoodNet surveillance population increased from 14.2 million persons in five sites to 41.5

million in nine sites (14% of the U.S. population).

FoodNet began active surveillance for laboratory-diagnosed cases of *Salmonella* in 1996. In 2003, there were 6,017 laboratory-diagnosed cases of *Salmonella*. Among the 5,455 (91%) *Salmonella* isolates serotyped, five serotypes accounted for 59% of infections: 1,104 (20%) Typhimurium, 759 (14%) Enteritidis, 653 (12%) Newport, 348 (6%) Heidelberg, and 331 (6%) Javiana. As in previous years, *Salmonella* infections affected children disproportionately. The incidence of *Salmonella* infection, defined as the number of laboratory isolations per 100,000 persons, was 122.7 for infants (i.e., aged <1 year) and 50.6 young children (i.e., aged 1-4 years), compared with 10.8 for other persons (i.e., aged ≥5 years).

From 1996 to 2003, the estimated incidence of *Salmonella* decreased 17% (95% CI = 26% to 7% decrease). The estimated incidence of the most common *Salmonella* serotype, *S. typhimurium*, decreased 38% (95% CI = 47% to 27% decrease). The incidence of the next most common serotypes, *S. enteritidis*, *S. newport*, and *S. heidelberg*, showed considerable variation by year and did not change significantly. The incidence of *S. javiana* increased 227% (95% CI = 66% to 546% increase) from 1996 to 2003; most of this increase occurred in Georgia. Although the incidence of *Salmonella* infection has declined, among the five most common *Salmonella* serotypes, only *S. typhimurium* demonstrated a sustained decline in incidence.

Salmonella infections are caused by many different *Salmonella* serotypes with different animal reservoirs; therefore, changes in overall incidence of *Salmonella* are influenced strongly by the most common serotypes and their reservoirs. Year-to-year variation in incidence can in part be attributed to outbreaks. Thus far in 2004, several large *Salmonella* outbreaks have occurred that reinforce the public health challenges that exist regarding human Salmonellosis. Outbreaks have included produce-associated *S. javiana* infections, multidrug resistant hamburger-associated and direct animal contact-associated *S. typhimurium* DT104 infections, and *S. enteritidis* infections associated with consumption of raw almonds. These outbreaks highlight the need for ongoing evaluation of reservoirs, sources of contamination, transmission routes and antibiotic resistance.

Salmonella remains as an important and ongoing burden to public health. It is estimated that there are 38.6 cases of *Salmonella* infection for each culture-confirmed case. Targeted control measures can be implemented in the future. On-farm prevention efforts should include reduction of egg contamination with *S. enteritidis* and preventing contamination of produce. Control of antibiotic use in food animals must be evaluated to address the serious problem of multidrug resistant *Salmonella*. Lastly, surveillance and epidemiological investigation of outbreaks by public health officials are critical to determine the reser-

voirs and risk factors for prevention of *Salmonella* infections.

Dr. John Braddy, FDA, presented an Update on FDA's Proposed Regulation: Prevention of *Salmonella enteritidis* in Shell Eggs During Production. FDA is proposing measures to prevent *Salmonella enteritidis* (SE) contamination of shell eggs during egg production. The motivation for this proposal is a farm-to-table risk assessment of SE in eggs, which identified implementation of on-farm prevention measures as a very important step that could reduce the occurrence of SE infections from eggs. While voluntary quality assurance (QA) programs for egg production have led to meaningful reductions in SE illnesses, these programs are not always uniformly administered or uniformly comprehensive in their prevention measures.

Moreover, the most recent data from CDC show that SE illnesses have essentially remained steady for the past several years. CDC estimated that 118,000 illnesses were caused by consumption of SE-contaminated eggs in 2001. Accordingly, FDA believes that further actions to improve egg safety—building upon the safe consumer handling labeling and egg refrigeration at retail rule of 2000—are the most effective way to achieve our public health goals of a 50% reduction in overall Salmonellosis and a 50% reduction in SE outbreaks by 2010.

The proposed rule's SE prevention measures include:

- Provisions for procurement of chicks and pullets;
- A biosecurity program;
- A pest and rodent control program;
- Cleaning and disinfection of poultry houses that have had an environmental sample or egg test positive for SE before new laying hens are added to the house;
- Refrigerated storage of eggs at the farm;
- Producer testing of the environment for SE in poultry houses—if the environmental test is positive, FDA proposes that egg testing for SE be undertaken, and that, if an egg test is positive, the eggs be diverted from the table egg market
- Identification of a person responsible for SE prevention at each farm;
- Recordkeeping requirements for environmental and egg sampling and testing and for egg diversion; and
- Exemptions: the proposed rule would not apply to producers who sell all of their eggs directly to consumers or producers with fewer than 3,000 laying hens. In addition, if a producer has 3,000 or more laying hens and all eggs at a farm are to be given a treatment that will achieve at least a 5-log destruction of SE or processed into egg products, then only the proposed refrigeration requirements would apply.

The regulation as proposed will have an expected annual cost of $82 million and prevent an expected 33,450 illnesses due to SE annually, at a cost of $2,450 per illness prevented. The proposed regulation will provide expected total annual benefits of $580 million resulting in $498 million in net benefits annually.

Dr. Kenneth Petersen, FSIS, gave a regulatory update on *Salmonella* performance standards for Dr. Barbara J. Masters, Acting Administrator, FSIS. Significant food safety advancements have been made in the past year. In 2003, FSIS issued new procedures for utilizing *Salmonella* performance standards as a verification tool for food safety. Under these new procedures, instead of waiting for two consecutive failures of tests to trigger an in-depth review of plant Sanitation Standard Operating Procedures (SSOP) and HACCP plans, reviews are initiated after any series of tests fails to meet a standard. Improvements to the in-depth review process have also been implemented, such as the inclusion of Enforcement, Investigative Analysis Officers and other HACCP-trained personnel, in conducting HACCP and sanitation verification reviews at those facilities displaying negative performance trends. This process and other science based initiatives, including strategies implemented to reduce *E. coli* O157:H7, have played a significant role in reducing the prevalence of *Salmonella* in raw meat and poultry regulatory samples. Out of the number of regulatory samples collected and analyzed by FSIS between January 1 and October 31, 2003, 3.6 percent tested positive for *Salmonella*, as compared with 4.29 percent in 2002; and 10.65 percent in 1998.

While these results are positive, eliminating foodborne illness is an evolving challenge. Through analysis and discussions with the scientific community, public health experts, and all interested parties, issues have been identified that need to be addressed to attain the next level of public health protection. A brief description of these challenges is provided below. The resulting strategies should help FSIS pursue its goals and accomplish its mission of reducing foodborne illness.

The first challenge is the need to anticipate/predict risk through enhanced data integration. One significant way in which this can be accomplished is by thoroughly analyzing data obtained from FSIS' regulatory sampling, as well as other sources of data, so as to discern trends and identify connections between persistence, prevalence and other factors, such as plant practices, seasonal variations and establishment size.

The second challenge is the need for improved application of risk into regulatory and enforcement activities. Food safety problems need to be documented as they occur, so that conditions may be analyzed and, if need be, corrected as appropriate. A better understanding of the prevalence and types of food safety failures could allow better assessment of how to best address them. Data regarding the causes of

food safety violations, either within a specific establishment or within a class of establishments, can be utilized in order to better focus attention one the greatest risks. In addition, it can provide us with a tool to determine enforcement trends by district and by circuit, which supervisors can use to determine whether enforcement actions are being consistently applied.

The third challenge is the need for improved association of program outcomes to public health surveillance data. We have seen notable advances in preventing foodborne illness, which have been attributed in part to the implementation of HACCP. However, there still is a need to determine how specific policies affect public health. Data that links foodborne illness outbreaks with specific foods needs to be connected with prevalence data of specific pathogens in specific foods. To complete the linkage with public health outcomes, a strong connection with human health surveillance data is needed. FSIS, together with our partners in public health, is working to accomplish this through FoodNet. By focusing on these initiatives FSIS will further advance food safety in the U.S. and abroad. For more information, please read *Fulfilling the Vision: Updates and Initiatives in Protecting Public Health* available on the FSIS website at www.fsis.usda.gov**.**

Dr. Kunho Seo, FDA, reported on the development of a real time polymerase chain reaction (PCR) assay for the rapid and specific detection of *Salmonella enteritidis* in pooled eggs, ice cream, and raw almonds associated with human *Salmonella* outbreaks.

An assay was developed for the specific detection of SE in eggs, using an application of the fluorogenic 5' nuclease assay (TaqMan). In this assay, a segment of the gene *sefA* specific to *Salmonella* group D strains such as SE was used. The amplification of the target gene products was monitored in real-time by incorporating a fluorescent dye-labeled gene-specific probe in the PCR reaction. This method correctly detected and distinguished SE from nearly 50 of non-group D *Salmonella* and other non-*Salmonella* strains. Detection of s*efA* gene was linear for DNA extracted from approximately 10^2 – 10^9 CFU/ml in PBS and 10^3 - 10^8CFU/ml in raw egg. In two trials, when applied to detection of SE in homogenized egg pools and compared with conventional culture methods, the newly developed PCR method yielded a 100% correlation with results obtained using a conventional culture method. However, the PCR method required only 2 days, compared to the 5 days required by the cultural method. The sensitivity of this assay was approximately less than 1 CFU per 600 g of egg pool. The real-time PCR assay proved to be a rapid, highly sensitive test for detection and quantification of low concentrations of SE in egg samples. When applied to direct detection and quantification of SE in ice cream, the real-time PCR assay was as sensitive as the conventional plate count method in frequency of detection, but populations of SE derived from

real-time quantitative PCR were one to three logs higher than provided by most probable numbers and colony-forming units obtained by conventional culture methods.

Dr. W. Douglas Waltman, Georgia Poultry Laboratory Network, presented an overview of past and future poultry *Salmonella* diagnostic methods for environmental testing. Prior to the late 1980's, *Salmonella* monitoring in poultry involved primarily looking for *S. pullorum* in the birds themselves, and occasional parathyroid salmonellae that may have been involved in clinical disease. With the emergence of *S. enteritidis* associated with eggs, the focus of *Salmonella* monitoring and detection switched from the bird to the bird's environment. With this switch came the realization that the media and methods for detection were inadequate for environmental samples. Major improvements in the isolation of *Salmonella* from environmental samples came in the early 1990's. The later 1990's saw an emphasis on the poultry carcass, and with it came a need for more rapid detection. Numerous rapid kits became available, and have been adapted to the various sample matrices, from environmental samples to carcass rinses.

The epidemiology of *Salmonella* and *Salmonella* infections has become of greater significance over these last several years. Even though there exist well over 2000 serotypes of *Salmonella*, still only a couple dozen make up the vast majority of serotypes found. Various typing methods have been developed to further divide and separate individual strains within a serotype, and these include biotyping, antimicrobial susceptibility, and phage typing. More recently the use of molecular techniques involving DNA fingerprinting have been used successfully.

Dr. Richard K. Gast, USDA-ARS Southeast Poultry Research Laboratory (SEPRL), Athens, Georgia, presented the results of experimental infection studies on the deposition of *Salmonella heidelberg* inside eggs. Since the mid 1980's, the production of internally contaminated eggs by chickens infected with *Salmonella enteritidis* has been an important source of human illness on several continents. In response to this problem, substantial public and private funds have been spent on detecting and controlling *S. enteritidis* infections in commercial laying flocks. Although *Salmonella* serotypes other than *S. enteritidis* are also commonly found in the housing environment of egg-laying flocks, these other serotypes have rarely been found inside eggs or implicated in transmitting egg-borne disease. However, CDC has recently reported a significant association between eggs or egg-containing foods and *S. heidelberg* infections in humans. Using an experimental infection model that has previously been applied to document the deposition of *S. enteritidis* inside eggs, the present study determined if several *S. heidelberg* isolates could colonize reproductive tissues and thereby gain access to the interior contents of eggs laid by infected

hens.

In each of two similar trials, three groups of 24 specific-pathogen-free laying hens were orally inoculated with doses of approximately 10^9 colony forming units (CFU) of either an *S. enteritidis* strain (which caused egg contamination in several prior studies) or one of four *S. heidelberg* isolates that were originally obtained from egg-associated human disease outbreaks. Fecal samples were collected from all hens at weekly intervals. Internal organ samples were removed from euthanized hens at one and three weeks post-inoculation. The contents of all eggs laid during the first three weeks after inoculation were also sampled. All samples were cultured to detect *S. enteritidis* or *S. heidelberg.*

All *S. enteritidis* and *S. heidelberg* strains colonized the intestinal tracts of most inoculated hens and were shed in the feces at similar frequencies. Likewise, all five *Salmonella* strains invaded to reach the livers, spleens, ovaries, and oviducts of inoculated hens, with no significant differences observed between strains for any of these tissues. All five *Salmonella* strains were isolated from the liquid contents of eggs laid by infected hens, although the *S. heidelberg* strains were found at lower frequencies (ranging from 1.1% to 4.5%) than was the *S. enteritidis* strain (7.0% for the two trials combined).

This study demonstrates that some strains of *S. heidelberg* possess the ability to colonize the reproductive tract of chickens and can thereby be deposited inside eggs laid by these birds. However, as all four *S. heidelberg* strains used in these experiments were already associated with egg-transmitted human disease, the overall frequency at which these abilities are distributed among other strains of this serotype is not certain.

Dr. Jean Guard Bouldin, USDA-ARS-SEPRL, the Sidney Kimmel Cancer Center, San Diego, CA, and the USDA-ARS, Antimicrobial Resistance Unit, Athens, GA, presented work on the genomic differences between *Salmonella enteritidis* PT4 and PT13a.

Salmonella enteritidis (SE) is the leading cause of human Salmonellosis in the world and it is currently the second leading cause in the United States. Its success as a pathogen correlates with an ability to contaminate the internal contents of eggs produced by infected hens that are otherwise healthy. Historically, the emergence of new phage types within regions is sometimes associated with an increased incidence of human illness. In general, a few phage types predominate, but they can be divided into PT4 and non-PT4 lineages. PT4 is related by expression of typing phage receptors to PT 6, 5, and 7. The non-PT4 lineage includes historically endemic strains within the United States such as 13a and 8. Recent advances in nucleic acid microarray technology now make it possible to directly compare PT13a *S. enteritidis* in DNA-DNA hybridization assays to the complete genomes of

Salmonella typhimurium LT2 and *Salmonella enteritidis* PT4 in order to detect how phage type correlates with genomic organization. They investigated how the PT4 and non-PT4 lineages compare by DNA-DNA hybridization, the sensitivity of DNA-DNA hybridization for detection of genomic differences between and within phage type, and whether DNA-DNA hybridization was suitable for studying small scale genomic differences that result in divergence of biology in subpopulations but not phage type.

They used a *Salmonella*-specific microarray that represented PCR amplified sequences from the annotated open reading frames (ORFs) in *S. enterica typhimurium* LT2 (STM) supplemented with annotated chromosomal ORFs from Typhi CT18 strain (STY) and Enteriditis PT4 (courtesy Sanger Center, United Kingdom), that are more than 10% divergent from Typhimurium. Overall STM genome coverage for the array is 96.6% (4,338 genes), overall coverage of the STY genome is 94.5% (4,348 genes), excluding plasmids.

Strains chosen for analysis were two prominent subpopulations of *Salmonella enterica* serovar Enteritidis that vary in their virulence potential. They have an observable difference in ribotype pattern. Wildtype (WT SE) *S. enteritidis* produces HMM LPS and it is associated with high incidence egg contamination following systemic injection. The second strain of *S. enteritidis* produces biofilm (BF SE) and it has enhanced oral invasiveness. High incidence egg contamination has occurred consistently after contact infection of hens when these two strains are combined.

Their results from DNA-DNA hybridization showed that so far, only bacteriophage related sequence differs between the two major phage lineages of *S. enteritidis* PT4 and PT13a, which is in agreement with previously published assessments of the Rowe and Ward typing system used to classify *S. enteritidis*. The only phage type known to express receptors that bind both sets of typing phages is PT1, which is not frequently encountered. It is possible that PT1 is inherently unstable, because of conflicting compatibilities between bacteriophages. These results indicate that DNA-DNA hybridization is a powerful tool for investigating epidemiology related to phage type of food borne pathogens. However, other types of analyses will be needed for characterization of the subpopulation biology of *S. enteritidis*. This is an important issue, because subpopulation biology rather than phage type has been found to be more important for generation of high incidence egg contamination.

Dr. Peter Holt of the USDA-ARS-SEPRL, presented work on the effect of prior infection with a different *Salmonella* serotype on *Salmonella enteritidis* infection in birds during molt. Previous work in their laboratory showed that *Salmonella enterica* serotype Enteritidis (*S. enteritidis*) infections were generally more severe in hens undergoing

molt via feed withdrawal and they have been examining various situations that may affect the course of the infection. Laying hens can be infected with a variety of *Salmonella* serotypes besides *S. enteritidis* during the lifetime of the flock. While the presence of a potential human pathogen is a source of concern for the producer, this same organism could have a beneficial effect as earlier studies had shown that different salmonellae will compete for the niche in the chicken gut. Four trials were conducted which examined the effect of prior infection with *S. typhimurium* (trial 1-3) or *S. muenchen* (trial 4) on a *S. enteritidis* infection during molt. Levels of *S. enteritidis* were significantly reduced in hens receiving either the *S. typhimurium* or the *S. muenchen* indicating the potential benefits of having non-*S. enteritidis* serovars in the flocks. To examine this aspect further, a fifth trial was conducted in which hens received an aerosol dose of the live attenuated *S. typhimurium* vaccine MeganVac1 on day 1 of molt and then infected with *S. enteritidis* on day 4. There was a numerical but not significant decrease in *S. enteritidis* levels in those hens receiving the Megan Vac1, indicating that the available live *Salmonella* vaccines show promise as an intervention strategy for reducing potential *S. enteritidis* problems during a molt.

Dr. Anna Catharina Berge, Veterinary Medicine Teaching and Research Center, University of California-Davis, California, presented a report on the use of antibiotic susceptibility patterns and pulse field gel electrophoresis (PFGE) to compare historic and contemporary isolates of multi-drug resistant *Salmonella enterica* subspecies *enterica* serotype Newport. Recently, multi-drug-resistant (MDR) *Salmonella enterica* subspecies *enterica* serotype Newport reemerged as a public and animal health problem. The antibiotic resistance of 198 isolates and the PFGE patterns of 139 isolates were determined. *Salmonella newport* isolates collected between 1988 and 2001 were included in the study. One hundred seventy-eight isolates were collected from the San Joaquin valley in California and came from dairy cattle clinical samples, human clinical samples, bulk tank milk samples, fecal samples from preweaned calves, and waterways. Twenty clinical isolates from humans from various regions of the United States were also included in the study. Resistance to 18 antibiotics was determined using a disk diffusion assay. PFGE patterns were determined using a single enzyme (XbaI). The PFGE and antibiogram patterns were described using cluster analysis.

Although the antibiotic resistance patterns of historic (1988 to 1995) and contemporary (1999 to 2001) isolates were similar, the contemporary isolates differed from the historic isolates by being resistant to cephalosporins and florfenicol and in their general sensitivity to kanamycin and neomycin. With few exceptions, the contemporary isolates clustered together and were clearly separated from the historic iso-

lates. One PFGE-antibiogram cluster combination was predominant for the recent isolates, which were taken from human samples from all parts of the United States, as well as in the isolates from California, indicating a rapid dissemination of this phenotypic strain. The data are consistent with the hypothesis that the emergence of MDR *Salmonella newport* is not simply an acquisition of further antibiotic resistance genes by the historic isolates but reflects a different genetic lineage.

Mr. Kevin Elfering, Minnestota Department of Agriculture (MDA), presented a case report on the use of PFGE technology to link an outbreak of *Salmonella enteritidis* from French Toast to a Layer Flock. In November 2003, the MDA and Minnesota Department of Health (MDH) investigated a foodborne illness outbreak related to *Salmonella enterica* serotype Enteritidis (*S. enteritidis*) and shell eggs. The investigation utilized trace-back procedures and PFGE technology in linking the outbreak to a large egg production/processing facility. A proposed but never finalized USDA-APHIS rule was used in guiding the farm investigation.

On November 13, 2003, MDH started to receive reports of *Salmonella* infections from school nurses in Minnesota. One reported case was a school cook the other was a student at a different area school who happened to work at a local restaurant. The following day, the MDH Public Health Laboratory confirmed the two reported cases as having *S. enteritidis*. Two additional isolates of *S. enteritidis* were also identified that day. All four isolates were indistinguishable by PFGE. This PFGE subtype was given the Minnesota designation SE1B1, the most common subtype in Minnesota. One of the newly identified cases was also from the same Minnesota county as the first two cases. An infection control practitioner (ICP) for the area hospital reported to MDH additional suspect cases seen at the hospital. Interviews of the confirmed cases and suspect cases by MDH staff revealed that they had all patronized the same restaurant. An investigation of the restaurant was initiated on November 17 by MDH Environmental Health (EH) specialists conducted an environmental assessment of the restaurant on November 17. The restaurant closed that day and remained closed until November 20 for cleaning, disinfection, disposal of food items and the conduction of an assessment of restaurant worker illness histories. MDH-EH specialists interviewed restaurant employees about recent gastrointestinal illness. All restaurant employees were asked to submit stool specimens for Salmonella testing. Employees who reported any gastrointestinal symptoms within the previous month, or who tested positive for *Salmonella* on their first specimen, were excluded from work until two consecutive stool specimens obtained at least 24 hours apart tested negative for *Salmonella*. Information gathered during routine interviews was reviewed by an MDH epidemiologist in order to identify other potential cases associated with eating at

the restaurant. Confirmed cases were defined as persons from whom *S. enteritidis* SE1B1 was isolated and who reported eating at the restaurant prior to symptom onset, or who worked at the restaurant. Probable cases were defined as persons who had diarrhea (defined as 3 or more loose stools in a 24-hour period) and fever and ate at the restaurant during the week prior to symptom onset, or who had diarrhea and ate at the restaurant with a confirmed case. Names of patrons who had eaten at the restaurant from October 20 to November 15 were obtained from credit-card receipts.

A case-control study was conducted to identify vehicles for infection. Officials from MDA investigated the source of the eggs and conducted laboratory analysis of environmental samples and of eggs remaining at the restaurant at the time of the investigation. MDA also conducted a trace-back investigation to identify the source of eggs used by the restaurant at the time of the outbreak. One supplier, (a small egg packer) was identified and as part of the trace-back investigation, an evaluation of egg-handling practices at the egg packer was conducted. Two farms were identified as the source of the eggs and producer interviews and environmental and manure drag samples were conducted.

This was an outbreak of *S. enteritidis* SE1B1 infections associated with eating at a Minnesota restaurant. The outbreak was identified through routine surveillance activities at MDH. Documented transmission to patrons of the restaurant occurred for more than two weeks. French toast was statistically implicated as a vehicle; however, multiple foods likely acted as vehicles for patrons. Shell eggs were confirmed as the ultimate source of *S. enteritidis* through trace back and environmental testing at the farm of origin. This investigation clearly shows the importance of using pulsed field gel electrophoresis technology in conducting a foodborne disease outbreak investigations. This technology enabled the investigators to rapidly identify the source flock.

Several deficiencies in food holding and preparation, such as inadequate refrigeration and potential for cross-contamination, were identified at the restaurant. These deficiencies likely contributed to the survival, proliferation and cross-contamination that led to the outbreak. Extensive *S. enteritidis* SE1B1 contamination was found at the source egg farm. Control measures, such as extensive testing and diverting eggs to pasteurization were implemented at the farm; however, a more effective rodent control program and adequate barn cleaning and disinfection must be implemented.

In 1993 the jurisdiction for conducting farm investigations related to *S. enteritidis* was shifted from USDA- APHIS to FDA. FDA has developed a guidance document patterned after the 1993 proposed APHIS rule for FDA investigators on how to conduct a farm investigation. However, nothing has been published as statute or rule regarding the di-

version of shell eggs. Since FDA's jurisdiction is limited to interstate commerce, it is important to have FDA codify this guidance document as many states adopt Federal rules by reference. Trace-back investigations can be important in confirming the vehicle in foodborne outbreaks. By identifying an infected flock, we were able confirm that the vehicle in this outbreak was shell eggs, and to were able to prevent further distribution of contaminated eggs. For this reason, trace-back investigations should not be reserved solely for outbreaks in which the vehicle has already been confirmed, but also should be considered as a tool to help confirm vehicles when epidemiologic methods suggest but cannot confirm a food vehicle.

Dr. Jerry Maiers, Fort Dodge Animal Health, Overland Park, KS, presented work on the *Salmonella enteritidis* protection of commercial layers when vaccinated with attenuated live *S. typhimurium* prior to and/or during molt. Induced molting is an important economic tool used in the egg industry to recycle aging laying hens for a second egg production cycle. A popular method to induce molt is to withdraw feed until a specific loss of body weight is achieved. Studies by Holt, et al have shown that inducing molt by feed withdrawal altered the immune responsiveness. Antibody responses remained largely unaffected while cell-mediated immunity was greatly compromised. Further studies have shown that the stress caused by removal of water and feed resulted in greater shedding and horizontal transmission of *Salmonella enteritidis* organisms when challenged. Vaccination of pullets during the growing period with either *Salmonella enteritidis* bacterins or live attenuated *Salmonella typhimurium* vaccines have been commonly used by the poultry industry to reduce the susceptibility to *Salmonella* infection during the lay cycle. More recent studies have evaluated the benefit of administering a live *Salmonella typhimurium* vaccine before molt to increase protection during the second lay cycle.

Commercial layer pullets were administered live attenuated *Salmonella typhimurium* vaccine (PoulvacST) twice within the first three weeks by coarse spray. All birds were then administered a *Salmonella enteritidis* bacterin (Poulvac® SE) by injection at 13 weeks of age.

Study 1. At 64 weeks of age, two weeks before feed restriction, birds were divided into two groups. Group 1 was administered Poulvac ST (ST) by coarse spray and Group 2 was left unvaccinated. The Group 2 birds not receiving the pre-molt vaccination were removed from the house while the live ST vaccine was administered to Group 1. The unvaccinated Group 2 birds were then returned to the house 48 hours post vaccination. Eggs and egg belts were sampled a few hours post vaccination and again the following day. The ST vaccine was recovered at a fairly low rate the same day of vaccination but not on the following day, suggesting the vaccine did not survive in the environment for an extended period. Both groups of birds were removed from

feed at 66 weeks of age and then transported from the commercial farm to research facilities at Fort Dodge, Iowa.

Birds were housed in isolator units at the research facility. Both groups were challenged at 67 weeks of age, or one week after feed restriction. Twenty-five birds per group were given an oral dose of 2.17 x 10^6 *S. enteritidis* (SE) phage type (PT) 13a organisms. As there were no unvaccinated layers on the trial farm, Specific Pathogen Free (SPAFAS SPF) layers were challenged and used as unvaccinated controls (Group 3). Protection was evaluated at seven days post-challenge by culturing four organ pools: the reproductive tract, internal organs, intestines, and cecae. Negative cultures indicated protection from the SE challenge. Results showed a live/killed vaccination program without a pre-molt Poulvac ST vaccination gave solid protection against a moderate level of SE PT13a challenge through the first production period, even beyond the point of feed restriction. The addition of a live ST vaccination prior to molt (Group 1) did not lower the already low incidence of SE recovery post challenge (Group 2).

Study 2. The second part of the study evaluated protection into the second production cycle in birds from the same farm. Five groups of birds were evaluated. Group 1 was given ST at both pre- and post-molt, Group 2 was vaccinated only at pre-molt and Group 3 was vaccinated at post-molt when hens were not laying eggs. Group 4 was not ST vaccinated at either time and Group 5 was SPAFAS SPF layers that served as unvaccinated controls at the time of challenge. Birds were then brought to the Fort Dodge research facilities and challenged during the second production cycle at 74 weeks of age. SE phage type 13a was administered orally to each bird at a dose of 3.70 x 10^8 (about 100-fold higher than the first study).

The results indicate the live/killed pullet program without ST boost at molt did not offer a significant level of protection against the much higher level of SE PT13a challenge used in the second study. The addition of ST vaccination pre-molt (Group 2) or both pre and post-molt (Group 1), significantly enhanced protection from SE during the second production peak. Although ST administered to hens while out of egg production (post-molt Group 3) did not offer a significant level of organ protection, recovery rates of SE from the reproductive tract, intestines and ceca were similar to Groups 1 and 2.

Kathleen Kauffman of the New York State Cattle Health Assurance Program (NYSCHAP) and the New York State Animal Health Diagnostic Laboratory, Cornell University, College of Veterinary Medicine, Ithaca, NY, reported on the NYSCHAP and associated *Salmonella* Disease Module's Best Management Practices for preventing bovine Salmonellosis. The NYSCHAP is an integrated disease prevention program that utilizes a team of advisors to develop a farm-specific herd health plan. The objectives of this integrated herd health plan are to increase

the herd's health, productivity and profitability; assure food safety, public health, and consumer confidence in dairy and beef products; and promote environmental stewardship.

An advisory team can help develop management schemes that appropriately address complex issues faced by today's cattle producers. One of the strengths of NYSCHAP is the strong emphasis on this cooperative 'team' approach to develop and implement the health assurance program. Program success requires active participation from the producer, herd veterinarian, nutritionist and consultants.

The following steps are taken to design the herd health plan:

1. Define farm goals and areas of concern;
2. Assess health risks to the farm;
3. Develop the herd plan;
4. Review the herd plan with all farm personnel to ensure proper implementation; and
5. Producer and herd veterinarian review the herd plan quarterly and annual with the entire NYSCHAP team.

All farms must participate in the core module, which focuses on overall biosecurity and herd health issues regardless of specific disease or area on the farm. Additionally, producers can participate in specific disease modules, one of these being Salmonellosis. The Salmonellosis module has a specific risk assessment to examine the risks of introduction of disease and spread within the farm. For farms already experiencing outbreaks there is a more detailed risk assessment. Educational materials are available for this module on the NYSCHAP web site, www.nyschap.vet.cornell.edu.

Dr. Ed Mallison, University of Maryland at College Park, MD, spoke on the topic of airflow at the litter/manure surface: a key HACCP consideration. A close relationship has been repeatedly seen between *Salmonella*-positive litter surfaces on broiler farms and *Salmonella*-positive carcasses at processing while *Salmonella*-negative carcasses have been related to *Salmonella*-negative litter surfaces. Follow-up studies seeking to understand why some litter surfaces were positive and others spontaneously negative have revealed that stagnant to low airflow velocities at the litter/manure surface were associated with an increased risk for *Salmonella* contamination (high prevalence rates and high *Salmonella* counts) while higher velocities over such surfaces were associated with a reduced risk for *Salmonella* contamination (zero to very low prevalence rates and, when present, low *Salmonella* counts). These observations support their contention that proper airflow at the litter/manure surface, or other areas of accumulating fecal wastes, is a promising on-farm, pre-harvest critical *Salmonella* control point (JAVMA, 2001, Vol. 218, No. 12, pgs. 1919-1922).

Airflow and the reduction of *Salmonella* risks were related to a commonly used technique for suppressing bacteria, the control of free,

gaseous molecular water or water activity/availability (AW). Bacterial populations dramatically increase when levels of free water molecules, in or near a substance, are high. Conversely, bacterial populations collapse when these levels are reduced. Depriving bacteria from water molecules essential to their survival and multiplication (Aw control) is routinely used, for example, in quality assurance in paper manufacture, by the pharmaceutical industry, and in food preservation and packaging. In food science, water-binding salts and sugars, drying and water-blocking metallic foils are employed to make molecular water, essential to a bacterium's metabolism, unavailable.

Recent field studies have confirmed earlier investigations indicating that proper airflow over litter/manure surfaces is another practical way to reduce the availability of molecular water to bacteria. We have found that where ambient relative humidity was 70 to 80%, a modest drying airflow of 2 or more mph, *three* inches over the litter/manure surface, reduced both *Salmonella* isolations and counts. We also found that, in instances where ambient relative humidity was 90 to 100%, an airflow of 4 or more mph produced the same desirable effect.

Their results suggest that ventilation systems should be designed and operated to ensure that all litter/manure surfaces in a production facility receive proper minimal levels of airflow. Since *Salmonella* can be introduced into the food animal environment from previous flocks or herds, vermin, feed, hatcheries and parent stock, the provision of proper airflow appears to be a promising way to neutralize such introductions whenever or wherever they may occur. Collaborative research with agricultural engineers merits high priority to further explore the various parameters of this opportunity.

The Committee had an extensive discussion about the newly proposed FDA *Salmonella enteritidis* program. The following comments were recorded during the committee discussion:

Members stated that laboratory capacity varies by region and analytical standards to make labs eligible for participation have not been specified in the proposed rule. The possible role of state and federal laboratories (NVSL) and private laboratories remains unknown. The issues of laboratory standards and costs is uncertain.

A question was raised about the overall strategy, scope of the plan in relation to other federal salmonella programs. Dr. Braddy encouraged all parties to submit comments voicing questions and concerns to the public docket.

Committee members stated that states should conduct inspections and maintain ongoing relationships with egg producers. In addition, members felt that what FDA will accept as a "biosecurity" program may not be comprehensive enough in relation to existing quality assurance programs for example, pertaining to rodent control. In addition, training of farm personnel and related costs were of concern. Dr. Braddy

stated that training would be similar to that of the seafood HACCP alliance program including regional satellite programs, classes and courses.

Members voiced concern that producers and states will bear the major costs of the proposed rule since this is an unfunded mandate. Dr. Braddy stated that he was not able to respond to economic components of the plan. He stated that the average cost of the proposed rule is $20,000.00 per farm based on the 4,100 farms impacted by the proposed rule. Members stated that States do not charge producers to maintain existing voluntary egg quality assurance programs.

One member asked whether the requirement to test birds between 40-45 weeks of age was science-based and derived from a peer-reviewed study. Dr. Braddy stated that he was unfamiliar with the source of that data but that he would be willing to follow up on the question. In addition concern was voiced related to finding places to divert eggs to when a positive egg sample is detected. Some mentioned that current pasteurization capacity is limited. Others voiced concern wondering if customers would accept SE positive eggs for pasteurization and whether demerits would make marketing a financial burden to small producers. There does not seem to be enough surge capacity in the system to accommodate the proposed rule. One member asked whether indemnification is available if the flock must be destroyed in cases where egg diversion is not economically feasible or possible. Dr. Braddy encouraged members to submit such questions to the public docket.

Members noted that requirements on public health side to train food handlers has not kept pace with the important changes about to occur at farm level and that adoption and implementation of the FDA's model food code is consistent among States.

At the conclusion of the discussion, the Chair appointed a subcommittee to prepare written comments to FDA's public docket regarding the proposed rule affecting *Salmonella enteritidis* in shell eggs.

In addition, because the Committee has a vested interest in monitoring *Salmonella* Performance Standards, the Chair appointed a smaller subcommittee to deal with this issue over the coming calendar year.

Two resolutions were approved by the Committee and forwarded to the Committee on Nominations and Resolutions for approval by the general membership. The resolutions addressed:

1. Continuation of funding for the continuation of the molecular characterization of *Salmonella* field isolates for the NPIP program by NVSL.
2. Using a rigorous science-based approach to further developing *Salmonella* performance standards, for making these standards informal and non-regulatory, and that a secure data repository be developed to promote further analysis.