Report of the Committee on *Salmonella*

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Vice Chair: Douglas Waltman, Oakwood, GA

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The Committee met from 12:30 p.m. to 6:00 p.m. October 15, at the Minneapolis Hilton Hotel in Minneapolis, Minnesota. A total of 42 members and guests were in attendance. Dr. Patrick L. McDonough, new Chair, and new Vice Chair, Dr. Doug Waltman, presided.

Regulatory, Industry and Subcommittee Reports

Dr. Doug Waltman, Georgia Poultry Laboratory, presented the National Poultry Improvement Plan report, in place of Andy Rhorer, who was unable to attend. The report is included in these proceedings.

Ms. Brenda Morningstar-Flugrad, National Veterinary Services Laboratory (NVSL), Veterinary Services (VS), Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), presented the NVSL Report regarding *Salmonella.* The full text of the report is included in these proceedings.

Dr. Liz Wagstrom, National Pork Board, presented a report on the National Pork Board initiatives to minimize *Salmonella*. *Salmonella* contamination of meat is a growing concern for the pork industry. It has been estimated that 1.4 million cases of nontyphoidal *Salmonella* occur each year in the United Stated and about 1.3 million are thought to be foodborne. Also, *Salmonella* is a leading cause of food-borne diarrhea in humans. More than 2,500 *Salmonella* serotypes have been identified. Food animals may be infected with these organisms and act as potential sources for meat contamination. *Pork Checkoff* has funded many pre and post-harvest *Salmonella* research projects. Pre-harvest research projects have focused on issues such as defining prevalence levels, identifying risk factors and assessing interventions. One critical finding was the confirmation that both market pigs and cull sows may become rapidly infected with *Salmonella* while in abattoir holding pens. Also, a number of different interventions were evaluated for their potential to reduce *Salmonella* levels in the live pig. The approach varied from administering vaccines to providing various products to the live animal. The goal was to identify methods that would significantly reduce the risk of *Salmonella* shedding near the time of slaughter. This information could have a significant impact on the development of any pre-harvest

Salmonella reduction plan. Post-harvest research projects focused on topics such as identifying, evaluating and validating procedures that help reduce or eliminate bacterial contamination. A study found that chilling carcasses is a critical step in the reduction of bacteria. A comparison study that evaluated several different chilling methods identified temperature ranges and conditions which led to decreased performance. Another project identified several time and temperature parameters that small processors may use to help define their food safety plan. The objective of post harvest research is to provide valid scientific data that can help food processors improve the wholesomeness and safety of the pork products they harvest and process.

Dr. Scott J. Wells, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, presented Beef and Dairy Initiatives to reduce *Salmonella* in Cattle. Three different studies were presented to show how potential control methods are being explored and discovered to control manure-cycle pathogens on dairy farms. The USDA-APHIS-VS National Animal Health Monitoring System Dairy 2002 Study showed that the larger the size of a dairy herd, the more likely it was to be *Salmonella* positive. Also, the percentage of cows shedding *Salmonella* increased slightly between Dairy '96 and Dairy 2002 from 5.4% to 7.3%. In the next study (Fessler et al. 2004 JAVMA, 225:567-573) on the prevalence of *Salmonella* spp. on conventional and organic dairy farms, 129 farms were sampled up to five times at 2-month intervals after enrollment; enrollment was done without regard to previous history of salmonellosis. Results showed that 91% of farms had at least one cattle fecal sample positive for *Salmonella*, 47% of farms had at least one environmental sample positive for *Salmonella*, and 25% of farms accounted for 75% of positive samples. This study showed that while *Salmonella* is widely found on farms it tends to occur in clusters. The final study described herd-level risk factors associated with *Salmonella* on dairy farms (Fossler et al. 2005. Prev. Vet. Medicine 70(3-4):257-277), i.e., Summer (reference = winter), not storing purchased protein feeds/concentrates in enclosed building, not using monensin in weaned calf or bred heifer diets, manure disposal by slurry application or irrigation on owned or rented land, applying manure to fields that are harvested or grazed during same growing season, not using tiestall/stanchion housing for lactating cows, and cattle access to surface water. Once risk factor associations could be made, hypotheses may be developed and control programs can ultimately be designed. Dr. Wells stressed that we need verified control programs in order to successfully manage salmonellosis on farms.

Dr. David Dargatz, APHIS-USDA, provided an overview on the National Antimicrobial Resistance Monitoring System (NARMS) Report, in place of Dr. Paula Fedorka-Cray. The full report is included in these proceedings.

Dr. Richard Gast, Agricultural Research Service (ARS), USDA, presented the ARS Egg Safety and Quality Research Unit Report. Early in 2005, the USDA Agricultural Research Service created a new research group at the Russell Research Center in Athens, Georgia. The Egg Safety and Quality Research Unit (ESQRU) was formed by combining scientists and support personnel from other local ARS groups who had a common interest in research on this important agricultural commodity. The stated mission of the new group is "to protect both the health of consumers and the marketability of eggs by conducting research to develop improved technologies for egg production and processing that reduce or eliminate microorganisms that can transmit disease to humans or cause spoilage." Among the specific objectives of this research are determining how microbial pathogens infect poultry and cause egg contamination, understanding how poultry production practices can influence such infections, developing effective methods for preventing infection of egg-laying poultry by pathogens and for testing to detect infected flocks and contaminated eggs, and improving egg processing practices to reduce microbial contamination while enhancing egg quality. The new ESQRU has three primary research projects funded by ARS:

1.) Controlling Egg Contamination with *Salmonella enterica* by Understanding its Evolution and Pathobiology: Dr. Jean Guard-Bouldin (Lead Scientist), Dr. Richard Gast (Research Leader);

2.) Stress Effects on Immunity and Physiology of Poultry: Dr. Peter Holt (Lead Scientist), Dr. Randle Moore; and

3.) Egg Processing Safety, Quality, and Security: Dr. Deana Jones (Lead Scientist), Dr. Michael Musgrove.

Some of the early research results generated by this new group include (1) identification of bacterial properties of some *Salmonella* strains that enable them to cause egg contamination in infected chickens; (2) demonstrating that prompt refrigeration is important to prevent rapid multiplication of *Salmonella* that can migrate into the nutrient-rich contents of egg yolks; (3) showing that molting hens by feed withdrawal causes physiological changes in the gut and increases susceptibility to *Salmonella* infection but alternative methods for molting induction do not have this effect; and (4) demonstrating the effectiveness of current commercial egg washing practices for removing *Salmonella* from egg shells and showing that washing in cooler water may provide an alternative method more compatible with the need for rapid egg refrigeration.

One of three Subcommittee reports was presented. Dr. McDonough gave a brief overview of the Committee, encouraged members to take a copy of the AAVLD Abstract Program Booklet. *Salmonella* Performance Standards Subcommittee Report, due to a scheduling conflict, was not presented. The Ohio Egg Quality Assurance Program was presented in lieu of the Subcommittee Report.

The Ohio Egg Quality Assurance Program (OEQAP) report was presented by Dr. Tony Forshey, Acting Ohio State Veterinarian. For the purpose of enhancing food safety, reducing risk to public health and maintaining consumer confidence in Ohio produced eggs, the Ohio Poultry Association (OPA), in cooperation with the Ohio Department of Agriculture (ODA) and the Ohio Department of Health (ODH), has developed the following Ohio Egg Quality Assurance Program (OEQAP). Voluntary participation by the industry is a commitment to minimize the risk of *Salmonella enteritidis* (SE) in shell eggs. The program does not guarantee the eggs to be free of SE. Participants in the plan must implement and document the placement of SE monitored chicks; cleaning and disinfection procedures; rodent, fly and pest control programs; biosecurity measures; feed sourcing under a SE reduction plan; flock health monitoring program; and SE environmental and egg testing. The publication A Health Manual for Ohio Poultry Producers, produced by the Ohio State University Extension (OSUE), may be used as a template for developing written Best Management Practices (BMPs). OEQAP participants agree to develop and implement a program for their facility that includes the standards identified in the OEQAP. Review of participant plans will be made by the Ohio Department of Agriculture, as a third party verification of the plan. At a minimum, participants of the OEQAP agree to: production processing distribution, and food safety.

Dr. Eric Gingerich, New Bolton Center Poultry Laboratory, School of Veterinary Medicine, University of Pennsylvania gave the *Salmonella enteritidis* Subcommittee Report. The report was approved by the Committee and is included in these proceedings.

Salmonella Diagnostic Methods Subcommittee Report was not available for presentation, as Dr. Dr. Kakimba Nagaraja could not attend the meeting.

Dr. Robert O'Connor, Foster Farms, provided a report on *Salmonella* Performance Standards**,** from the perspective of the broiler industry. First he discussed the background of industry performance and FSIS initiatives. From 1998 until 2005 there has been an upward trend in *Salmonella* positives in the A set *Salmonella* testing. In February of 2006 FSIS introduced 11 initiatives to help the broiler industry in reversing the upward trend of *Salmonella* positives. Three performance categories were established to guide the use of FSIS resources. There has been concern to link human illness to a point of process. Next Dr. O'Connor discussed initiatives at the "live production" area; they determined that the "Chiller" water was the key to controlling *Salmonella* in the processing plant area. Then the processing plant interventions were presented. Key findings from this study include: chiller management (CO2/Cl2) and scalder overflow can directly impact final *Salmonella* levels in combination, need to consider effectiveness of interventions in combination and alone; changes should

be introduced in a controlled and systematic way; instinct needs to be supported with information. Impact at the breeder level extends throughout the value chain – rewards from success early in the value here are high.

Human Health Perspective

Dr. Elaine Scallan, Enteric Diseases Epidemiology Branch, FoodNet, presented an update from the Centers for Disease Control and Prevention (CDC): Human *Salmonella* Trends. The full text of this report is included in these proceedings.

Dr. Gerardo A. Ramirez, Office of Plant and Dairy Foods, Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), gave an update on FDA's current priorities: Identification of Research Needs Relating to *Salmonella enteritidis* Contamination of Eggs. This report is included in these proceedings.

Dr. Ken Petersen, Food Safety Inspection Service (FSIS), gave the FSIS Update on *Salmonella* Performance Standards, which provided a progress report on *Salmonella* testing of raw meat and poultry products 1998-2005: The full text of his update is included in these proceedings.

Diagnostics

Dr. John Maurer, Poultry Diagnostic and Research Center, University of Georgia, presented Monophasic *Salmonella typhimurium*. The report included information on *Salmonella* 4,(5), 12:I:- monophasic serotype that has been recognized in the United States. Cattle, wild birds, and poultry have been colonized with this serotype. The question is whether *Salmonella* 4, (5), 12:I:- is a Typhimurium? In order to answer this question, the genotype of *S. enterica* O4,[5],12; i; - and Typhimurium were compared. *S. enterica* O4,[5],12; i;- has the virulence plasmid of Typhimurium and the virulence genes on Typhimurium's prophage. So is *S. enterica* O4,[5],12; i;- a Typhimurium? Yes, and it should be considered virulent, too. Is *S. enterica* O4,[5],12; i;- a clone? No, since it is distributed amongst the strains of Typhimurium. Next the genetic basis for the *S. enterica* O4,[5],12; i;- phenotype was discussed, i.e., it is proposed that the fljB promoter is lost for the phase II of *S. enterica* O4,[5],12; i;- or the entire phase II fljB gene is actually lost. Then the issue of what is driving the emergence of *S. enterica* O4,[5],12; i;- was discussed, e.g., the use of vaccines in hosts?, or the occurrence of a phage?, or some other unknown cause.

Pathobiology

Mutational Mapping and Location of Single Nucleotid Polymorphisms in *Salmonella enteritidis* Isolates that Vary in Virulence was presented by Dr. Jean Guard Bouldin, Egg Safety and Quality Research Unit, ARS-USDA. The report included whole genomic sequencing and mutational mapping to compare strains of *Salmonella enteritidis*. The full text of this report is included in these proceedings.

Dr. Kyle Newman, Venture Laboratories, presented Effects of Mannan Oligosaccharide on Antibiotic Resistance Expression in *Salmonella*. The use of sub-therapeutic levels of antibiotics in animal feed is falling out of favor in a number of countries that are concerned about resistant bacteria in animal production systems infecting humans. In addition, developing next-generation antimicrobial agents has not been considered a priority for pharmaceutical companies due relatively low profit margins. For this reason, decreasing the prevalence of antibiotic resistant bacteria has become an important research area. Antibiotic resistance in bacteria can be passed from one organism to another through a variety of mechanisms and decreasing the prevalence of antibiotic resistant bacteria has become an important research area. A study was undertaken to investigate the prevalence of antibiotic resistance in a multiple antibiotic resistant strain of *Salmonella* (plasmid mediated) and methods to cure that resistance using Mannan oligosaccharide (MOS). In *in vitro* systems the presence of Streptomycin-resistant strains of *Salmonella* was eliminated in the presence of 0.3% mannan oligosaccharide (control, 100% resistant; MOS, 0% resistant). The same organism also carried resistance to ampicillin. The

presence of ampicillin resistance in the untreated control was 100%. Exposure of the culture to MOS decreased the prevalence of ampicillin resistance to 40.3% in the presence of 0.3% MOS and 15.1% in the presence of 0.5% MOS. In a swine feces slurry, conjugation (antibiotic resistance transfer from one organism to another via plasmid transfer) was significantly diminished in the presence of 0.3% MOS. The implications are that materials (MOS) can be introduced into animal diets without toxicity or residue concerns and reduce the concentrations of antibiotic resistant bacteria in the animal would benefit the industry.

Epidemiology and Field Reports

Dr. Beth Mamer**,** University of Idaho, Department of Animal and Veterinary Science, Caine Veterinary Teaching Center, presented *Salmonella* Case Reports in Cattle – Two *Salmonella* Cases in Cattle: A Microaerophilic *Salmonella montevideo* from a Fetus and *Salmonella*-contaminated Milk Replacer. The first case is the report on a *Salmonella enterica* serovar Montevideo isolated from a mummified, four month in gestation bovine fetus from a large Holstein dairy with an increase in abortions. The fetus was necropsied and samples submitted to the Microbiology section for bacterial culture and Fluorescent antibody detection of viruses and protozoa. The only significant findings from this fetus were pure cultures of numerous pinpoint Beta hemolytic colonies on Columbia blood agar from liver and stomach contents after two days in capnic culture. After three days in culture, a second set of pinpoint Beta hemolytic colonies was isolated. Both isolates were eventually identified as *Salmonella enterica* serovar Montevideo. These isolates grew readily in an anaerobic chamber. We could identify these isolates with media and kits that are normally used to isolate and identify members of *Enterobacteriaceae* with prolonged incubation and/ or oil overlay of the media.

The second case is the report on four *Salmonella enterica* serovars isolated from two-week-old bull calves at a calf raising operation. The healthy calves arrived at one day of age to the calf raiser. These calves were fed milk replacer and then calf starter. Over a three-week period, the one-week-old calves would show rapid onset of diarrhea and die. At the beginning of the outbreak we tested tissue samples from four calves that had died in two days. From each calf we isolated a different serovar of *Salmonella enterica*. These serovars included: C2-Newport; G-Havana; K-Cerro; and, D-Dublin. We suggested the calf raiser look at environmental contamination because more than one serovar was identified. They submitted feed samples from both the current and the previous weeks feed samples, which included milk replacer, calf starter and alfalfa pellets. We isolated *Salmonella enterica* serovar Havana only from the previous week's milk replacer. The calf raiser changed milk replacer companies. Over the next two weeks they still lost more calves to diarrhea and death. We were able to isolate serovars Havana and Dublin from these calves. The new milk replacer tested negative for *Salmonella* species.

Kevin Elfering, Minnesota Department of Agriculture, presented Case Report of *Salmonella* in Poultry Meat - Questionable Labels and Confusing Products *Salmonella enteritidis* and *Salmonella typhimurium* Outbreaks Associated with Frozen Chicken Entrees, Minnesota, 2005-2006. The full text of this report is included in these proceedings.

Dr. Rude Hein, Intervet Inc., provided an update of *Salmonella enteritidis* and *Salmonella* Trends in the European Union, including information on *Salmonella enteritidis* and *S. typhimurium* control in the European Union (EU) in layers and breeding stock. He provided links to information on the topic at three URL's:

- European Commission. Reducing *Salmonella*: Commission sets EU targets for laying hens and adopts new control rules. Press release. 1 August 2006. (http://europa.eu/rapid/pressReleasesAction.do?reference=IP/06/1082&type=HTML&aged=0&language=EN&guiLanguage=en);

- COMMISSION REGULATION (EC) No 1168/2006 of 31 July 2006 implementing Regulations (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of Gallus gallus and amending Regulation (EC) No 1003/2005 (Text with EEA relevance) (http://eurlex.europa.eu/LexUriServ/site/en/oj/2006/I_211/I_21120060801en0040008.pdf)
- European Food Safety Authority (EFSA). Preliminary report: Analysis of the baseline study on the prevalence of *Salmonella* in laying hen flocks of Gallus gallus. 14 June 2006. (http://www.efsa/eu/int/science/monitoring_zoonoses/reports/1541/zdc_*Salmonella*_report_ej81_layinghens_en1.pdf).

Recently revised rules to control programs for breeding stock in member states and non-EU countries (export to EU) were implemented (January 2007). The rules cover *Salmonella enteritidis, Salmonella typhimurium, Salmonella hadar, Salmonella infantis, Salmonella virchow.* EU member states may have national control programs that have rules that go beyond target EU rules and that impose additional import controls.

Approval to export poultry or hatching eggs into the EU require: a formal request and meeting the EU requirements, completing a questionnaire for the EU – Export Country, inspections may be conducted, based on results specific conditions may be discussed with member states, and finally if all is okay the proposal to import would be allowed.

A European Food Safety Authority (EFSA) baseline study on the prevalence of *Salmonella* in laying hens was conducted, i.e., June 2006 (EFSA Journal 2006 81,1-71). This survey was conducted from September 2004 – October 2005 among all Member states in flocks:> 1,000 birds (holding). Two pooled dust / 5 pooled feces samples were taken during the last 9 weeks of their production period. 5317 laying flocks tested (holdings) were tested. Results showed 20.3% positive for *Salmonella enteritidis/typhimurium* (SE/ST); percent positive varied from 0 -62.5%; 30.7% positive for all *Salmonella spp.*; and varied from 0 -79.5%. SE/ST isolations in laying hens for the time period 2004-2005 were detailed for member states with more than 1 million layers. (EFSA preliminary report June 2006), in addition more frequently isolated *Salmonella* serotypes were detailed.

The present annual targets for layer flocks (2006-2007) were presented: 10% reduction (preceding year:< 10%), 20% reduction (preceding year:10 -19%), 30% reduction (preceding year: 20 - 39%), 40% reduction (preceding year: >40%). The ultimate target remains <2% or lower (2009 – 2010). Member states with a prevalence of SE/ST of 10% or more by January 2008 have to vaccinate. Similar targets already set for breeders, and separate targets exist for broilers/ turkeys will be set in coming years. Antimicrobials should not be used (exceptions). Trade ban will be imposed, i.e., no eggs sold, if there are *Salmonella* positive flocks (proposal 2010).

Vaccination guidelines for laying hens and breeders were provided: Vaccines authorized by EU/National Governments, live vaccines (SE/ST must be differentiated from field strains by July 2007), layers - mainly live breeders - increasing inactivated vaccines, some countries no live vaccines (France), increase use of vaccination due increase of free range birds, broilers not vaccinated, no Autogeneous vaccines will be allowed.

Vaccination guidelines for broilers were also provided: no vaccination, EU control programs are in preparation (January 2009), and member states may have national control programs; sampling will occur the week before processing (i.e., "shoe sampling"), sampling processing will involve "neck skin."

A Time-Specific paper, A Field Study of *Salmonella* Prevalence on Swine Farms, was presented by Dr. David Dargatz. The complete paper is included in these proceedings.

Intervention Strategies

Dr. Hailu Kinde, California Animal Health and Food Safety Laboratory System (CAHFS), gave the Committee and update on California Egg Quality Assurance Program Trends—Environmental Monitoring of *Salmonella enterica* serovar Enteritidis (SE) in Commercial layer flocks in California

(1991-2006). Testing of manure or egg machinery in a poultry houses is a practical and cost effective method for screening the environment for SE and should be used as one indicator of the effectiveness of the intervention strategies for reducing SE in eggs. Developing a "one- size- fits- all" program for environmental sampling of layer houses is a challenge because of the vast number of variations in styles or types of layer houses. For example, just considering only different types of manure collection/disposal systems, these may include high-rise deep pit, shallow pit, manure belt, shallow pit flush and cage-free floor systems. Many of the Egg Quality Assurance Programs specify the manure-type sample as the sample of choice (e.g. The Pennsylvania Egg Quality Assurance Plan (PEQAP), the California Egg Quality Assurance Plan (CEQAP), and the Ohio Egg Quality Assurance Plan (OEQAP). However, there are others that prefer the egg machinery-type sample (e.g. UEP 5-Star) or a combination of the two (Maine). Programs that require testing at the end of lay have proven efficacious in reducing both environmental contamination and human disease. In California, producers submit at least one set of samples during each lay cycle. The number of samples collected needs to be practical and take into consideration the cost of testing, while adequately assessing the presence of SE in the house. The various programs recommend different numbers of samples depending on the size and type of house. The PEQAP recommends collecting 2 manure drag swabs per row/bank. The OEQAP requires 2 drag swabs per row and then pools the 2 swabs from a row. The CEQAP recommends collecting a standard number of 16 manure swabs from each house regardless of the house type and to pool these samples into 4 samples of 4 swabs each. The 16 swabs were calculated using a binomial distribution model assuming 10% of the drag swab area was contaminated with SE. Based on this assumption, the use of 16 swabs gives an 81% certainty of detecting SE with a confidence level of 95%. This method has served well and accepted by the California egg industry uniformly.

This paper analyzes data on the proportions of *Salmonella* and SE detected from environmental manure drag swabs and chick papers of commercial layer flocks in California from 1991 to 2006. Overall the proportion of positive isolation of *Salmonella* from drag swabs during the 16 years of testing has an upward trend of 14.5% (1991) to 50% (2006). This dramatic increase in the detection of *Salmonella* is largely due to the implementation of the Delayed Secondary Enrichment (DSE) method since 1995. DSE has been proven to increase the detection rate of *Salmonella* by 30 to 40% over the primary enrichment method. For the same period of time however, the trend for SE has decreased from 5.5% (1995) to 3.8% (August 2006).

Dr. Eric Gingerich, New Bolton Center Poultry Laboratory, School of Veterinary Medicine, University of Pennsylvania provided the Committee with an update on Pennsylvania Egg Quality Assurance Program Trends (PEQAP). He presented 10 years of progress in controlling *Salmonella enteritidis* (SE) in table egg layers. PEQAP was developed in 1995 out of a federal program, the SE Pilot Project that studied the epizootiology of SE in table egg layers in Pennsylvania. From that project, a set of best management practices and a monitoring program were set forth in an effort to aid producers in their attempts in reducing SE. This presentation showed the progress made over the years in reducing the number of flocks positive for SE and hence the number of SE positive eggs produced. For example, the percentage of SE positive flocks has declined from 38% in 1992 to 7% in 2005 and the percent SE positive drag swabs has declined from 23% in 1992 to less than 1% in 2005.

Discussion, Resolutions and Recommendations

No Resolutions were discussed from the Committee members or from the non-members present during the meeting. Dr. McDonough stated that during the coming year he would network with all members to ascertain the needs and goals for their stakeholder industries. Also, we need to work with USDA-APHIS-VS-NVSL *Salmonella* Serotyping Laboratory to develop a letter to send to laboratories that have not been providing serotyping test results to VS, and in that way we hope to ensure that the VS serotyping results are more representative of what we are finding nationally in the United States. We also need to keep appraised of *Salmonella* research activities nationally and internationally so that we

may apply results to our respective disciplines related to *Salmonella.* During the year each of our Subcommittees will remain active and their activities will be shared with the Committee membership. There is a need for the United States to develop an internationally accepted scheme to fingerprint *Salmonella* serotypes occurring here from animal/avian sources and to share these findings, so that we may apply these results to our respective animal industries; such molecular epidemiological findings will have direct application to prevention, control, and to public and animal health as we tract infections domestically and internationally.

The National Poultry Improvement Plan (NPIP) Report

Doug Waltman
Georgia Poultry Laboratory

In Calendar Year 2005, there were 2 isolations /outbreaks of *Salmonella* Pullorum reported to the Poultry Improvement Staff. There was one isolation/outbreak of *Salmonella* Pullorum reported during Calendar Year 2006 from January to October 1, 2006. There have been no isolations of *Salmonella* Gallinarum since 1988 in any type poultry. The isolates in 2005 were all standard strains of *Salmonella* Pullorum and the isolate in 2006 was an intermediate strain. The number of birds in *Salmonella Pullorum* positive flocks (January 1, 2005- October 1, 2006) was as follow:

Number of Birds	No. of Flocks	Strain of Pullorum
>5<25	1	Standard
>25<50	1	Standard
>200	1	Intermediate

Hatchery Participation in the National Poultry Improvement Plan Testing Year 2005	
Egg and Meat-Type Chickens: Participating	283
Capacity	698,974,826
Turkeys Participating	49
Capacity	33,285,723
Waterfowl, Exhibition Poultry and Game Birds	721
Capacity	26,321,162

Egg-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year 2005	
U.S. Pullorum-Typhoid Clean: Participating- Number	184
Birds in Flocks-Number	3,914,294
Average per Flock	21,273
Primary Breeding Flocks Flocks – Proportion of Total	21.7
Primary Breeding Flocks Birds- Proportion of Total	12.2

Meat-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year 2005	
U.S. Pullorum-Typhoid Clean: Participating- Number	4,866
Birds in Flocks-Number	76,744,870
Average per Flock	15,772
Primary Breeding Flocks Flocks-Proportion of Total	9.7
Primary Breeding Flocks Birds-Proportion of Total	6.5

Turkey Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year 2005	
U.S. Pullorum-Typhoid Clean: Participating –Number	525
Birds in Flocks-Number	4,009,155
Average per Flock	7,636
Primary Breeding Flocks Flocks-Proportion of Total	20.6
Primary Breeding Flocks Birds-Proportion of Total	3.8

Waterfowl, Exhibition Poultry, and Game Birds Breeding Flocks In the National Poultry Improvement Plan Participation and Testing Summary Testing Year 2005	
U. S. Pullorum-Typhoid Clean Participating	3,649
Birds in Flocks	1,173,993
Primary Breeding Flocks Flocks-Proportion of Total	34.9
Primary Breeding Flocks Birds- Proportion of Total	58.1

***U.S. Salmonella enteritidis* Clean - Egg-Type Chickens**
No. of flocks and birds in the flocks with *Salmonella enteritidis* isolates, 1990-2006

	Environmental	Dead Germ	Bird
Flocks	56	6	19
Birds in Flocks	599,871	77179	201,342

U.S. *Salmonella enteritidis* Clean- Egg-Type Chickens
No. of flocks and birds in flocks by State with *Salmonella enteritidis* isolates, 1990-2006

Arkansas	Environmental	Dead Germ	Bird
Flocks	1		15000
Birds in Flocks	6000		2
Georgia			
Flocks	1	2	
Birds in Flocks	400	46000	
Illinois			
Flocks	3	2	1
Birds in Flocks	3900	3700	1200
Indiana	Environmental	Dead Germ	Bird

U.S. *Salmonella enteritidis* Clean- Egg-Type Chickens
No. of flocks and birds in flocks by State with *Salmonella enteritidis* isolates, 1990-2006

Flocks	15	2	1
Birds in Flocks	158345	27479	15092
Kentucky			
Flocks	1		
Birds in Flocks	6625		
Ohio			
Flocks	14		9
Birds in Flocks	183700		91600
Oregon			
Flocks	2		
Birds in Flocks	19516		
Pennsylvania			
Flocks	14		6
Birds in Flocks	166385		78450
Texas			
Flocks	1		
Birds in Flocks	10000		

Phage type13	Environmental	Dead Germ
Flocks	10	2
Birds in Flocks	143000	3700
Phage type 13A		
Flocks	5	2
Birds in Flocks	54321	27479
Phage type 2		
Flocks	2	
Birds in Flocks	28900	
Phage type 23		
Flocks	21	
Birds in Flocks	16,000	
Phage type 28		

Flocks	2	2
Birds in Flocks	15000	46000
Phage type 34		
Flocks	2	
Birds in Flocks	12500	
Phage type RNDC		
Flocks	1	
Birds in Flocks	7000	
Phage type Untypable		
Flocks	2	
Birds in Flocks	24000	
Phage type 8		
Flocks	15	
Birds in Flocks	157701	

Egg-type Chicken breeding flocks with isolates of *Salmonella enteritidis* by phage type and by year 1989-2006

Year	No. Flocks	Phage Type
1989	1	13A
1990	11	13A, 13, 8, 28
1991	12	13A, 13, 8
1992	10	Untypable,13A,8,28,34
1993	5	Untypable, 8, 2
1994	3	13A, 8
1995	2	13A, 28
1996	5	Untypable, RNDC, 13A,8,2
1997	2	8
1998	2	8
1999	1	13
2000	4	13, 8
2001	1	13
2002	0	
2003	0	
2004	0	
2005	1	13
2006	1	34

The National Veterinary Services Laboratory Report

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Serotyping results for 16,737 *Salmonella* isolates from animals and epidemiologically related sources are reported for July 1, 2005 through June 30, 2006. The most frequently identified serotypes were *Salmonella Typhimurium, S. Heidelberg, S. Kentucky, S. Newport* and *S. Anatum.*

Salmonella isolates submitted by animal disease diagnostic laboratories throughout the United States are received at the National Veterinary Services Laboratories (NVSL) for serotyping. The *Salmonella* are isolated from cases of clinical disease and from herd and flock monitoring. Data are also included on *Salmonella* isolated by the Food Safety and Inspection Service as a result of HAACP testing. Data generated from the serotyping of research isolates are not included in this report. There are two tables presenting serotype information by source: one from cases of clinical disease and one table presenting serotypes by source data from monitor samples, environmental samples, feed, and those listing "other" as the clinical role.

NVSL did not receive any information from other laboratories serotyping Salmonella over the past year. Because we have not received this information, this report will not be as complete as in previous years. We would encourage other laboratories serotyping *Salmonella* isolates of animal origin to resume sending information to NVSL to be included in the annual USAHA summary. No identifiers about the origin of the isolates are needed other than the state and animal species of origin and whether the isolate came from a clinical case or surveillance study.

The World Health Organization (WHO) Collaborating Centre in the format of the Kauffmann-White scheme follows the serotype information for Reference and Research on *Salmonella* and the Centers for Disease Control and Prevention (CDC). The Subspecies designation precedes the antigenic formula for those serotypes other than subspecies I. Those serotypes previously reported as "Arizona" are now listed with "III" (both monophasic and diphasic) followed by the antigenic formulae. Those serotypes belonging to subspecies II or IV that had been previously named are now listed with their antigenic formula preceded by II or IV. *Salmonella* Java is now named *S. paratyphi* B var. L-tartrate+. Group E_2 and E_3 serotypes are now designated by the E_1 serotype name followed by "var. 15+" or "var. 15+, 34+".

Serotyping results are presented for 16,737 *Salmonella* isolates, a 6.5% decrease over the 17,951 isolates reported last year.[1] This year 44% of the isolates were from clinical cases and 56% were from monitor samples, compared to 42% and 58% last year, repectively.[1] Of the clinical isolates, 50% were of bovine origin and 24% were isolated from swine. Thirty-four percent of the monitor samples were isolated from chickens and 20% were recovered from turkeys.

A total of 268 serotypes were identified from isolates recovered from animals, their environment, or feed in 41 states and the District of Columbia. The 10 most common serotypes (Table 1- ***all Tables are found in Appendix A at the end of this report; Tables 3 and 4 were not part of the report***) accounted for 61% of the total isolates reported. Table 2 lists the 10 most common serotypes by clinical role: those from clinical cases and those from monitor samples. *Salmonella typhimurium, S. heidelberg, S. agona*, and *S. montevideo* are found in both lists.

Salmonella typhimurium was again the most frequently identified serotype from all sources and clinical roles. (Table 1) It was the most common serotype from clinical cases and the third most common serotype from monitor samples (Table 2). *Salmonella typhimurium* was among the five most frequently identified serotypes isolated from chickens, cattle, swine and horses (Tables 5, 7, 8, and 9). Sixteen percent of all isolates, 22% of isolates from clinical cases, and 11% of isolates from monitor samples were identified as *S. typhimurium*, compared to 18%, 22%, and 11%, respectively, last year.[1] Fifty-seven percent of the *S. typhimurium* isolates were identified as *S. typhimurium* var. copenhagen

this year, compared to 52% last year.[1] The majority of *S. typhimurium* isolates recovered from swine were *S. typhimurium* var. copenhagen (84%); while 40% of *S. typhimurium* isolates of chicken origin, and 11% of those of equine origin were *S. typhimurium* var. copenhagen.

An untypable serotype 4,5,12:i:- increased to 437 this year from 274 last year[1], 95 in 2004[2], and 164 in 2003.[3] One hundred-twenty-five of these were isolated from chickens, 81 from cattle, and 37 from horses. This serotype was among the five most common serotypes from equine clinical cases (Table 10). This serotype is believed to be *S. typhimurium* that has lost the ability to express the phase 2 flagellar antigen.

Salmonella newport was the fourth most frequently identified serotype from all sources (Table 1) and second in clinical cases (Table 2). It was the second most common serotype from clinical cases in cattle (Table 7) and accounted for 13% of the isolates of bovine origin. *Salmonella newport* was the second most common serotype from clinical cases in horses (Table 9) and accounted for 15% of the isolates of equine origin. Five percent of the total isolates from all sources and all clinical roles were *S. newport*, compared with 9% last year[1], 8% in 2004[2], and 8% in 2003.[3]

Salmonella enteritidis was identified more frequently than any year since 2000 (Table 1). Forty-five percent of the isolates were of chicken origin and it was the most frequently identified serotype from chicken clinical cases and the fifth most common serotype from chicken monitor samples (Table 5). Nineteen different phage types were identified among the 271 *S. enteritidis* isolates that were phage typed. The most frequently identified phage types were type 13 (30%), type 8 (45%), and type 22 (5%).

Twenty-five different phage types were identified among 522 *S. typhimurium* isolates that were phage typed. The most common phage types were DT104 (36%) and U302 (19%). Fourteen percent were untypable.

Table 1. *Salmonella* Serotypes Identified Most Frequently From July 1, 2005 through June 30, 2006 with Comparison Data for 5 Years (All Sources, All Clinical Roles)

Serotype	2006	2005	2004	2003	2002	2001
Typhimurium**	3223 (1)	3211* (1)	2256 (1)	2810 (1)	2760 (2)	3862 (1)
Heidelberg	1668 (2)	1436(3)	826 (3)	2454 (2)	3043 (1)	3382 (2)
Kentucky	1651 (3)	1360 (4)	740 (4)	1425 (4)	1203 (4)	803 (5)
Newport	1060 (4)	1609 (2)	920 (2)	1522 (3)	1271 (3)	978 (3)
Anatum	860 (5)	352 (12)	197 (13)	469 (10)	454 (9)	495 (9)
Montevideo	847 (6)	579 (7)	276 (10)	718 (7)	1025 (5)	742 (6)
Agona	836 (7)	549 (9)	380 (7)	644 (8)	613 (7)	858 (4)
Senftenberg	821 (8)	734 (5)	667 (5)	749 (5)	937 (6)	703 (7)
Hadar	758 (9)	682 (6)	560 (6)	472 (9)	382 (11)	432 (12)
Derby	611 (10)	569 (8)	344 (8)	737 (6)	366 (12)	469 (10)

* NUMBER OF TIMES SEROTYPE WAS IDENTIFIED
** INCLUDES *S. TYPHIMURIUM* AND *S. TYPHIMURIUM* VAR COPENHAGEN
( ) RANK BEGINNING WITH THE MOST COMMON

TABLE 2. MOST COMMON SEROTYPES, ALL SOURCES, 7/05-6/06

Clinical			Monitor	
Typhimurium	1630		Kentucky	1402
Newport	698		Heidelberg	1372
Agona	492		Typhimurium	1052
Montevideo	363		Hadar	673
Orion var 15+ 34+	362		Senftenberg	592
Anatum	313		Enteritidis	391
Heidelberg	239		Montevideo	274
Derby	236		Agona	251
Dublin	219		4,5,12:i:-	213
Muenster	194		Cerro	212
All Others	2689		All Others	2870
Total	7435		**Total**	9302

TABLE 5. MOST COMMON SEROTYPES, CHICKENS 7/05-6/06

Clinical			Monitor	
Enteritidis	41		Heidelberg	908
Typhimurium	23		Kentucky	563
Kentucky	22		Typhimurium	231
Heidelberg	20		Senftenberg	196
III 40:z4,z23:-	8		Enteritidis	181
All Others	47		All Others	1040
Total	**161**		**Total**	**3119**

TABLE 6. MOST COMMON SEROTYPES, TURKEYS 7/05-7/06

Clinical			Monitor	
Senftenberg	64		Hadar	567
Heidelberg	29		Senftenberg	327
Montevideo	15		Heidelberg	131
Hadar	10		Schwarzengrund	78
Agona	7		Saintpaul	77
Bredeney	7			
All Others	41		All Others	638
Total	**173**		**Total**	**1818**

TABLE 7. MOST COMMON SEROTYPES, CATTLE 7/05-6/06

Clinical			Monitor	
Typhimurium	606		Cerro	159
Newport	494		Kentucky	56
Orion var 15+34+	362		Anatum	52
Montevideo	281		Newport	51
Agona	129		Montevideo	49
All Others	1682		Orion var 15+34+	49
All Others	158			

Total	**3705**		**Total**	**574**

TABLE 8. MOST COMMON SEROTYPES, SWINE 7/05-6/06

Clinical			**Monitor**	
Typhimurium	624		Typhimurium	137
Derby	222		Derby	59
Choleraesuis (kunzendorf)	149		Agona	16
Heidelberg	137		4:5:12:i:-	7
Agona	84		Choleraesuis (kunzendorf)	6
All Others	550		All Others	36
Total	**1766**		**Total**	**261**

TABLE 9. MOST COMMON SEROTYPES, HORSES 7/05-6/06

Clinical			**Monitor**	
Typhimurium	201		**Total**	**17**
Newport	122			
Agona	94			
4,5,12:i:-	34			
Javiana	27			
Total	**822**			

TABLE 10. MOST COMMON SEROTYPES, DOG/CAT 7/05-6/06

Clinical	
Newport	30
Typhimurium	13
Montevideo	4
Enteritidis	4
All Others	54
Total	**89**

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National Antimicrobial Resistance Monitoring System Report

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"Update: The National Antimicrobial Resistance Monitoring System – Enteric Bacteria (NARMS): Animal Arm" (Paula J. Fedorka-Cray, J. Stan Bailey, Jonathan G. Frye, Charlene R. Jackson, Mark D. Englen, Mark Berrang and Richard Meinersmann: Richard Russell Research Center, USDA-ARS, BEAR, Athens, GA; Nora E. Wineland and David A. Dargatz: USDA-APHIS-VS, NCAHS, Fort Collins, CO; Acknowledging the contributions of Neena Anandarama, Office of Public Health Science, USDA, FSIS, Washington, DC.

Antimicrobial susceptibility testing remains an important tool as investigators devise ways to arrest the development of antimicrobial resistance, particularly in food borne bacteria. In 1996, the Food and Drug Administration (FDA) initiated the National Antimicrobial Resistance Monitoring System - Enteric Bacteria (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. Isolates recovered from humans, food animals and retails meats are included in the program. The animal arm of NARMS resides at the USDA-ARS laboratory in Athens, GA while the human arm resides at the CDC in Atlanta, GA and the retail arm resides at the FDA-OR in Laurel, MD. Careful analysis of data is warranted as antimicrobial resistance varies between and within the different serotypes of *Salmonella*. Use of the information will be targeted to redirecting drug use to diminish the development and spread of resistance.

Introduction

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, an antimicrobial resistance monitoring program was proposed by the Food and Drug Administration Center for Veterinary Medicine (FDA). 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 CDC initiated the National Antimicrobial Resistance Monitoring System - Enteric Bacteria (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; NARMS currently monitors antimicrobial susceptibility in non-typhoid *Salmonella, Escherichia coli, Campylobacter,* and *Enterococcus* in humans and animals. The NARMS program was expanded to include testing of *Salmonella typhi*, *Listeria*, *Vibrio* and *Shigella* isolates collected from humans and isolates from retail meat. The animal arm of NARMS resides at the USDA-ARS laboratory in Athens, GA while the human arm resides at the CDC in Atlanta, GA and the retail arm resides at the FDA-OR 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. Information may be accessed at

http://www.fda.gov/cvm/narms_pg.html. Additional information on results from the animal arm of NARMS can be found at http://www.ars.usda.gov/Main/docs.htm?docid=6750.

Materials and Methods. Isolates

Slaughter**:** Samples were collected at federally inspected slaughter and processing plants as part of the HACCP (Hazard Analysis and Critical Control Point) Program. Samples were processed according to culture procedures described in the FSIS Microbiology Laboratory Guidebook (MLG)[1].

Diagnostic: Isolates were randomly selected from those submitted to the National Veterinary Services Laboratories, Ames, IA. Diagnostic isolates were also submitted to NARMS by participating veterinary diagnostic laboratories serving as sentinel sites. Current participating sentinel sites include Florida, Indiana, Iowa, New York, Oklahoma, Pennsylvania, Tennessee, Washington, and Wisconsin.

Susceptibility Testing

Antimicrobial susceptibility testing was done using a semi-automated system (Sensititre™, TREK™ Diagnostics, Inc., Cleveland, Ohio) according to manufacturer's recommendations. Clinical and Laboratory Standards Institute (CSLI; formerly known as the National Committee for Clinical and Laboratory Standards (NCCLS)) guidelines were followed throughout the testing procedure.

Results and Discussion.

The top serotypes by source for *Salmonella* slaughter isolates (1997-2005) are shown in Table 1. These data highlight the wide variability of serotype prevalence between animal sources. The association of serotype with a particular animal species may be associated with host or other unknown factors as is observed for *S.* choleraesuis var. kunzendorf, the host-adapted serotype of swine[2]. Other factors affecting serotype distribution include clinical status of the host and regional and seasonal collections[3]. While some overlap is observed for serotype distribution and rank for isolates recovered from ill or dead animals, marked differences are noted from those recovered from presumed healthy animals presented for slaughter (data not shown).

Table 1. Top serotypes by Source for *Salmonella* slaughter isolates (1997-2005)

	SOURCE							
Rank	**Cattle n=6813**		**Chicken n=10,620**		**Swine n=3,848**		**Turkey n=3,097**	
	Serotype	**%**	**Serotype**	**%**	**Serotype**	**%**	**Serotype**	**%**
1	Montevideo	13.9	Kentucky	35.5	Derby	25.8	Heidelberg	20.9
2	Anatum	8.9	Heidelberg	20.3	Typhimurium var. 5-[a]	11.2	Hadar	16.6
3	Newport	7.6	Typhimurium var. 5-[a]	6.2	Infantis	6.5	Senftenberg	8.1
4	Muenster	7.1	Typhimurium	4.9	Johannesburg	6.3	Reading	7.3
5	Typhimurium	5.6	Enteritidis	4.3	Anatum	6.1	Saint Paul	6.5
6	Typhimurium var. 5-[a]	5.5	Hadar	4.0	Reading	4.0	Agona	5.0
7	Kentucky	5.1	Monophasic	3.1	Heidelberg	3.9	Schwarzengrund	4.5
8	Mbandaka	4.0	Montevideo	2.7	Saint Paul	3.0	Muenster	3.7
9	Cerro	3.9	Thompson	2.3	Typhimurium	2.8	Arizona	2.7
10	Agona	3.8	Schwarzengrund	2.2	Agona	2.7	Typhimurium	2.5

[a]Formerly referred to as *S. typhimurium* var. copenhagen

The development of antimicrobial resistance also appears to be serotype dependent and is affected by the clinical status of the animal species from which it is recovered, although there are exceptions[3]. In general, resistance to more antimicrobials is observed for isolates originating from diagnostic sources while less resistance can be observed for the same serotype if the isolate originated from a slaughter or on-farm (i.e. healthy) source. The variation between resistance, regardless of food animal species or source, is shown in Table 2. In general, multiple antimicrobial resistance is observed more often for serotypes Typhimurium, Typhimurium var. 5-, Heidelberg and Newport, with Newport exhibiting the most resistance. *Salmonella enteritidis* exhibits the least resistance among the serotypes shown and in general among all serotypes. It is interesting to note that resistance most often occurs to the historical drugs (antimicrobials that have been in use the longest), sulfamethoxazole, tetracycline, and streptomycin. Further, there is a marked difference between resistance to a number of antimicrobials for both Typhimurium and Typhimurium var. 5-. This suggests that it would not be appropriate to include the variant as a 'general' Typhimurium as resistance to some antimicrobials may be underrepresented while resistance to others may be overrepresented. This is of significance particularly when comparing data between different monitoring systems, as may be done between the animal[4], human[5], and retail[6] arms of NARMS.

Table 2. Percent resistance among serotypes from all food animal sources for 2004

2004	SEROTYPE (%R) [all food animal sources]						
ANTIMICROBIAL	**Kentucky N=635**	**Typhimurium var. 5- N=441**	**Typhimurium N=387**	**Heidelberg N=306**	**Newport N=305**	**Derby N=229**	**Enteritidis N=113**
Amoxicillin/ Clavulanic Acid	8.7	20.2	16.3	15.0	73.4	8.3	1.8
Ampicillin	10.1	74.4	53.0	22.2	76.4	10.9	4.4
Cefoxitin	8.7	19.5	15.8	15.0	72.1	8.3	1.8
Ceftiofur	8.7	19.5	17.8	15.0	73.4	8.3	1.8
Ceftriaxone	0	0.9	0.5	0.7	4.9	0	0
Chloramphenicol	0.8	47.8	43.2	9.8	72.1	5.2	0.9
Gentamicin	1.9	6.6	8.8	13.4	16.4	7.0	1.8
Kanamycin	0.5	19.0	15.2	25.8	28.5	7.0	0.9
Nalidixic Acid	0.2	1.1	2.8	0	1.3	0	0
Streptomycin	31.2	61.5	53.2	32.7	78.7	60.7	4.4
Sulfizoxazole	3.9	78.2	58.4	21.6	77.7	59.8	2.7
Tetracycline	37.8	75.7	56.8	39.5	79.0	70.7	4.4
Trimethoprim/ Sulfamethoxazole	0.2	5.0	9.3	3.6	4.3	1.7	0

Use of this information will be targeted to redirecting drug use to diminish the development and spread of resistance. Since the information generated from NARMS, or any monitoring system, is descriptive only and does not address attribution and/or etiology of observed changes, outbreak investigations and field studies will be initiated as a result of major shifts or changes in resistance patterns in either animal or human isolates. Data from this type of research will fill known information

gaps and clarify observational discrepancies. Additionally, NARMS isolates are invaluable for other research areas including development of diagnostic tests, the study of molecular mechanisms of resistance, gene flow and population genetics, and for virulence and *in vivo* colonization studies.

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Salmonella enteritidis Subcommittee Report

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The FDA Proposed Rule for *Salmonella enteritidis* was presented – the Proposed Rule was announced on September 2004; the Comment period lasted until December 2004; the FDA reopened the Comment Period for additional comments for more input on pullets in May 2005. The final rule is expected sometime in 2007, and is at the FDA general council now; next it goes to the HHS then OMB; the final rule will state the timeframe for implementation.

Update from CDC: Human *Salmonella* Trends

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Adapted from: MMWR April 14, 2006 55(14):392-395. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food – 10 States, United States, 2005: This report described preliminary surveillance data for 2005 and compares them with baseline data from the period 1996 to 1998. Foodborne illnesses are a substantial health burden in the United States (1). The Foodborne Diseases Active Surveillance Network (FoodNet) of CDC's Emerging Infections Program collects data from 10 U.S. states* regarding diseases caused by enteric pathogens transmitted commonly through food. FoodNet quantifies and monitors the incidence of these infections by conducting active, population-based surveillance for laboratory-confirmed illness (2). Incidence of infections caused by *Campylobacter*, *Listeria*, ***Salmonella***, Shiga toxin-producing *Escherichia coli* O157 (STEC O157), *Shigella*, and *Yersinia* has declined, and *Campylobacter* and *Listeria* incidence are approaching levels targeted by national health objectives (3). However, most of those declines occurred before 2005, and Vibrio infections have increased, indicating that further measures are needed to prevent foodborne illness.

In 1996, FoodNet began active, population-based surveillance for laboratory-confirmed cases of infection from *Campylobacter*, *Listeria*, *Salmonella*, STEC O157, *Shigella*, *Vibrio*, and *Yersinia*. In 1997, FoodNet added surveillance for cases of *Cryptosporidium* and *Cyclospora* infection. In 2000, FoodNet began collecting data on STEC non-O157 and comprehensive information on hemolytic uremic syndrome (HUS). FoodNet personnel ascertain cases through contact with all clinical laboratories in their surveillance areas. During 1996--2005, the FoodNet surveillance population increased from 14.2 million persons (5% of the U.S. population) in five states to 44.5 million persons (15% of the U.S. population) in 10 states. Preliminary incidence for 2005 was calculated using the number of laboratory-confirmed infections and dividing by 2004 population estimates. Final incidence for 2005 will be reported when 2005 population estimates are available from the U.S. Census Bureau.

2005 Surveillance

In 2005, a total of 16,614 laboratory-confirmed cases of infections in FoodNet surveillance areas were identified, as follows: *Salmonella* (6,471 cases), *Campylobacter* (5,655), *Shigella* (2,078), *Cryptosporidium* (1,313), STEC O157 (473), *Yersinia* (159), STEC non-O157 (146), *Listeria* (135), *Vibrio* (119), and *Cyclospora* (65). Overall incidence per 100,000 population was 14.55 for *Salmonella*, 12.72 for *Campylobacter*, 4.67 for *Shigella*, 2.95 for *Cryptosporidium*, 1.06 for STEC O157, 0.36 for *Yersinia*, 0.33 for STEC non-O157, 0.30 for *Listeria*, 0.27 for *Vibrio*, and 0.15 for *Cyclospora*. Substantial variation occurred across surveillance sites. Of the 5,869 (91%) *Salmonella* isolates serotyped, six serotypes accounted for 61% of infections, as follows: Typhimurium, 1,139 (19%); Enteritidis, 1,080 (18%); Newport, 560 (10%); Heidelberg, 367 (6%); Javiana, 304 (5%); and a monophasic serotype identified as *Salmonella* I 4,[5],12:i:-, 154 (3%). Among 109 (92%) *Vibrio* isolates identified to species level, 59 (54%) were *V. parahaemolyticus*, and 15 (14%) were *V. vulnificus*. FoodNet also collected data on 145 STEC non-O157 isolates that were tested for O-antigen determination; 117 (81%) had an identifiable O antigen, including O26 (37 [32%]), O103 (36 [31%]), and O111 (23 [20%]); 28 isolates did not react with the typing antisera used. In 2005, FoodNet sites reported 205 foodborne disease outbreaks to the national Electronic Foodborne Outbreak Reporting System; 121 (59%) were associated with restaurants. Etiology was reported for 159 (78%) outbreaks; the most common etiologies were norovirus (49%) and *Salmonella* (18%).

Editorial Note:

In 2005, compared with the 1996--1998 baseline period, significant declines occurred in the estimated incidence of *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, STEC O157, and *Yersinia*

infections. Several important food safety initiatives (*1*) might have contributed to the declines, indicating progress toward meeting the national health objectives (*3*). However, most progress occurred before 2005. Most of the decline in *Campylobacter* incidence occurred by 2001, with continued small decreases since then. The incidence of *Listeria* infections in 2005 is higher than its lowest point in 2002. Of the five most common *Salmonella* serotypes, only Typhimurium has declined, with most of the decline occurring by 2001. Most of the decline in STEC O157 incidence occurred during 2003 and 2004. The observed sustained increase in *Vibrio* incidence indicates that additional efforts are needed to prevent *Vibrio* infections. Oysters are the most important source of human *Vibrio* infections, and most human infections can be prevented by not eating raw or undercooked oysters. Measures that reduce *Vibrio* contamination of oysters also prevent illness.

Food animals are the most important source of human *Salmonella* infections. Transmission of *Salmonella* to humans can occur via various food vehicles, including eggs, meat, poultry, and produce, and via direct contact with animals and their environments. Testing by the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) at slaughter and processing plants has demonstrated declines in *Salmonella* contamination of ground beef since 1998 (*4*). However FSIS recently announced a sustained increase in chicken-broiler carcasses testing positive for *Salmonella* during 2002--2005 and subsequently launched an initiative to reduce *Salmonella* in raw meat and poultry products (*4,5*). Although sources of infection with the most common *Salmonella* serotypes have been identified (e.g., food animals), further investigation is needed to identify sources for emerging *Salmonella* serotypes, such as Javiana and I 4,[5],12:i:-, a monophasic serotype that resembles *S. typhimurium* except that it has no phase 2 flagellar antigen and has previously been misclassified as Group B *Salmonella* or *S. typhimurium* (*6*).

Large outbreaks with multiple laboratory-confirmed cases can distort underlying trends in incidence. For example, the incidence of *Cryptosporidium* infections increased substantially from 2004 to 2005 because of a large outbreak associated with visits to a recreational water park in New York (P Smith, MD, New York State Department of Health, personal communication, 2006).

The findings in this report are subject to at least four limitations. First, FoodNet relies on laboratory diagnoses, but many foodborne illnesses are not diagnosed by clinical laboratories. Second, protocols for isolation of certain enteric pathogens (e.g., STEC non-O157) in clinical laboratories vary and are not uniform within and among FoodNet sites (*7*); others (e.g., norovirus) cannot readily be identified by clinical laboratories. Third, reported illnesses might have been acquired through nonfoodborne sources, and reported incidence rates do not reflect foodborne transmission exclusively. Finally, although the FoodNet surveillance population is similar to the U.S. population (*2*), the findings might not be generalizable to the entire U.S. population.

Much remains to be done to reach the national health objectives for foodborne illnesses. Enhanced measures are needed to understand and control pathogens in animals and plants, to reduce or prevent contamination during processing, and to educate consumers about risks and prevention measures. Such measures can be particularly focused when the source of human infections (i.e., animal reservoir species and transmission route) are known. The declines in the incidence of STEC O157 infections observed in recent years suggest that coordinated efforts by regulators and industry have been effective in reducing contamination and illness related to ground beef (*8,9*).

Consumers can reduce their risk for foodborne illness by following safe food-handling recommendations and by avoiding consumption of unpasteurized milk and unpasteurized milk products, raw or undercooked oysters, raw or undercooked eggs, raw or undercooked ground beef, and undercooked poultry (additional information on food safety for consumers is available at http://www.fightbac.org). Other effective prevention measures, such as pasteurization of in-shell eggs, irradiation of ground meat, and pressure treatment of oysters, can also decrease the risk for foodborne illness.

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* Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and selected counties in California, Colorado, and New York.

Update on FDA's Current Priorities: Identification of Research Needs Relating to *Salmonella enteritidis* Contamination of Eggs

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The safety of shell eggs continues to be important to FDA. Working toward decreasing the number of egg associated *Salmonella enteritidis* (SE) cases remains a goal of FDA. In an effort to achieve this goal, FDA is currently in the process of developing an SE research plan. The primary goal of this research plan is to identify areas of further research need, relating to SE in eggs, and development of projects to address these needs. A preliminary search of the literature indicated several processes in the contamination of eggs by SE that are not fully understood. FDA has identified several of these areas as those needing to be investigated further.

In reviewing the literature base on SE research, it is apparent that differences exist within strains in their susceptibility or resistance to SE. The root of these differences should be investigated further in an effort to identify what causes increased resistance in certain strains. In addition to genetics of the layers, the exact steps in intestinal colonization by SE are not completely understood. It is known that adherence to the intestinal mucosa is a critical step in colonization of the gastrointestinal tract. Further research should be conducted to develop methods of preventing adhesion or altering the binding sites. The primary route of egg contamination with SE is through transovarian transmission (vertical transmission) yet this process is not fully understood. Research should be aimed at identifying all cells involved, regions of the reproductive tract where SE colonizes, cellular signals that account for intermittent laying of SE positive eggs, and whether colonization of the reproductive tract occurs in an ascending or descending manner. Another area of research need is the dynamics of SE contamination in a layer house. Few studies have closely scrutinized the spread of SE through the layer house and the dynamic interactions of all parameters involved. Although many of the individual parameters leading to increases in SE infections are known the progression of SE from the rodent reservoir, to the layers an ultimately into the eggs is not completely understood. Research should also be conducted on other serotypes of *Salmonella*. Data from CDC has identified an increase in *Salmonella heidelberg* infections. Attention should be paid to other serotypes that could potentially be transmitted through eggs. It is important to understand how SE makes its way from the environment and ultimately ends up within eggs. The better the understanding of all processes and parameters involved the more effective the reduction strategies that will be developed.

FDA maintains data on annual SE outbreaks, the latest data available being from 2005. In 2005 there were four SE outbreaks with all four being associated with eggs. The outbreaks occurred in a home setting, hospital and restaurant with the location of the fourth outbreak not being reported. These 4 outbreaks resulted in 129 cases and no deaths. The outbreaks involved six states including California, Georgia, North Carolina, Oregon, South Carolina and Washington. These four outbreaks represent a slight increase from the two outbreaks reported in 2004; however, they also represent a substantial decrease from the 15 outbreaks that were reported in 2003.

FDA remains focused on insuring the safety of shell eggs. Identification of research need areas is an important step in assuring that safety. Collaboration with other agencies such as USDA-FSIS and USDA-ARS for the purposes of addressing further research needs and researching those needs already identified is planned for the near future.

FSIS Update on *Salmonella* Performance Standards

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The Food Safety and Inspection Service (FSIS) issued the Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule on July 25, 1996 (Federal Register, Vol. 61, No. 144, pp. 38805-38989). To verify that industry Pathogen Reduction/HACCP (PR/HACCP) systems are effective in controlling the contamination of raw meat and poultry products with disease-causing bacteria, the PR/HACCP rule sets *Salmonella* performance standards that slaughter establishments and establishments that produce raw ground products should meet. These product-specific limits on *Salmonella* became effective in large establishments on January 26, 1998, in small establishments on January 25, 1999, and in very small establishments on January 25, 2000. FSIS verifies that establishments are meeting the standards by directing federal inspection program personnel to collect randomly selected product samples and send them to FSIS laboratories for *Salmonella* analysis, according to procedures described in Appendix E of the PR/HACCP final rule (Federal Register, Vol. 61, No. 144, pp. 38917-38928).

The *Salmonella* performance standards are based on the prevalence of *Salmonella* as determined from the agency's nationwide microbiological baseline studies conducted before PR/HACCP was implemented. Raw products currently covered by performance standards are carcasses of cows/bulls, steers/heifers, market hogs, and broilers, and ground beef, ground chicken, and ground turkey. The performance standards are expressed in terms of the maximum number of *Salmonella*-positive samples that are allowed per sample set. The number of samples in a sample set varies by product, and the maximum number of positive samples allowed in a set provides an 80% probability of an establishment passing when it is operating at the standard.

There are two phases of the FSIS regulatory program for *Salmonella* in raw products: non-targeted and targeted testing. Non-targeted or "A" set tests are collected at establishments randomly selected from the population of eligible establishments that are not currently in the targeted phase of the program, with a goal of scheduling every eligible establishment at least once a year. Other codes (such as "B", "C", and "D") represent sample sets collected from establishments that are targeted for follow-up testing following a failed set. The scheduling for targeted testing is based on inspection program guidance provided in Chapter 3 of FSIS Directive 5000.1.

The data reported are from the non-targeted regulatory testing program, i.e., results from the code "A" samples. The Agency recognizes that some establishments having the most difficulty in controlling *Salmonella* can be in the targeted testing for an entire year, and are, therefore, not subject to non-targeted testing during that year. Nevertheless, in the absence of continuous baseline studies, the Agency considers the "A" set data to be the best set of data to indicate trends. The regulatory test results are also compared to the performance standards, which were based on microbiological baseline studies, determined prior to the implementation of PR/HACCP.

Initiative announced at a public meeting in Feb. 2006 and in the Feb 27, 2006 Federal Register. The initiative will include concentrating resources at establishments with higher levels of *Salmonella* and changes the reporting and utilization of FSIS *Salmonella* verification test results.

The Healthy People 2010 goal for foodborne illnesses associated with *Salmonella* is 6.8 illnesses per 100,000 people. In the most recent Centers for Disease Control and Prevention data released in April 2005, the incident rate was 14.7 per 100,000 people. Overall, *Salmonella* infections dropped 8 percent from the previous year, but only one of the five most common strains declined significantly.

Effort patterned after the highly successful FSIS initiative to reduce the presence of *E. coli* O157:H7 in ground beef. The FSIS *E. coli* O157:H7 initiative led to a 40 percent reduction in human

illnesses associated with the pathogen, according to the Centers for Disease Control and Prevention (CDC).

While overall, percentage of positives across seven testing categories has been falling, since 2002, FSIS has seen an increase in *Salmonella* positive samples in broilers. Although the overall percentage of positive samples in verification testing of broilers is still below national baseline prevalence figures of 20%, the recent upward trend is of concern to the Agency. 2002-11.5; 2003-12.8; 2004-13.5; 2005-16.3. (Rates for last two quarters was 14.5 and 12 percent.)

The strategy will include concentrating resources at establishments with higher levels of *Salmonella* and changes the reporting and utilization of FSIS *Salmonella* verification test results.

Under the strategy, FSIS will provide the results of its *Salmonella* performance standard testing to establishments on a sample-by-sample basis, rather than waiting for results from entire sets. The more rapid disclosure will allow establishments to more readily identify and respond to needed process controls in the slaughter and dressing operations.

FSIS will also post quarterly nationwide data for *Salmonella* on its Web site, conduct follow-up sampling sets as needed, and provide new compliance guidelines for the poultry industry. Following two completed sample sets, FSIS will categorize each establishment according to the percentage of positive *Salmonella* samples. Broiler plants will be listed in either category 1 (10% or less prevalence), category 2 (>10-20%) or category 3 (>20%).

FSIS is identifying the following human health related serotypes in broiler *Salmonella* sets – Heidelberg, Enteritidis, Montevideo, Newport, and Infantis. Data analysis since 1998 indicates that plants in category 3 are significantly more likely than plants in category to have *Salmonella* serotypes of human health concern in their *Salmonella* sets. The odds ratio for this is 6.2 (CI: 3-13).

In early 2007, FSIS will assess whether industry has reduced the *Salmonella* prevalence towards category 1. FSIS will consider further activities such as posting individual plant results on the web.

In summary, the FSIS goal is to decrease the prevalence of *Salmonella* in broiler carcasses to a preponderance of plants consistently operating in category 1. This goal will both support movement toward the healthy people 2010 objectives and reduce serotypes of human health concern in broiler sample sets.

Mutational Mapping and Location of Single Nucleotide Polymorphisms in *Salmonella enteritidis* Isolates that Vary in Virulence

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Salmonella enterica subspecies i-*enterica* serotype Enteritidis (*S. enteritidis*) is currently the leading cause of salmonellosis worldwide and the second leading cause in the United States. The Centers for Disease Control (CDC) and the USDA Food Safety Inspection Service (FSIS) have recently described epidemiological trends that suggest that this pathogen could be increasing in incidence in people and in broiler chickens. Research is needed to identify small scale genetic change that correlates with the ability of *S. enteritidis* to cause food borne outbreaks because methods such as DNA-DNA hybridization microarrays and pulsed field gel electrophoresis (PFGE) have failed to differentiate between strains that vary in virulence phenotype. The objectives of this project are to identify single nucleotide polymorphisms (SNPs) that differentiate the genomes of two isolates that were obtained from a single parent strain but that nonetheless had different pathological outcomes in laying hens.

To locate SNPs, mutational mapping was performed by comparative genome sequencing (CGS), which is a commercially available service (Nimblegen, Inc). CGS requires that a genomic database be available to generate overlapping primers that resolve sequence to a single base pair. Phage type (PT) 4 *S. enteritidis* genome sequence is available from the Pathogen Sequencing Group at the Sanger Institute (http://www.sanger.ac.uk/Projects/Salmonella/). DNA was extracted from three isolates of *S. enteritidis*, one of which was a PT4 isolate used as a template to generate the overlapping primers. The other two isolates submitted for CGS were PT13a isolates that varied in their ability to contaminate eggs. One of these isolates could contaminate eggs, grow to high cell density, and produce a capsular LPS molecule at 25°C and it was designated wt *S. enteritidis*. The other PT13a isolate was orally invasive, produced biofilm but could not contaminate eggs or grow to high cell density or produce much capsular LPS at 25°C. It was designated bf *S. enteritidis*. Both strains were descended from a single parent strain.

Results (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome&cmd=search&term=) were that 409 SNPs out of the 4.686 million base pairs in the genome, or less than 0.01% of the genome, differentiated the two PT13a isolates that varied in virulence potential. There was an average of 8.5 SNPs per 100,000 bp. Areas of the genome that had lysogenic phage could not be compared in this assay, because primers made to the PT13a specific bacteriophage Fels-2 were absent for lack of template in the PT4 genome and primers made to the PT4 specific phage, ST64b, were lacking target DNA in the PT13a strains. Thus, SNPs that occur within phage genes that differ between PT4 and PT13a strains are not included in the total and will require manual sequencing. The virulence plasmid from the two PT13a strains differed by 5 SNPs. All classes of genes had SNPs, although genes involved in metabolism were most heavily represented and included more than 30% of the genes identified. Curvilinear analysis of SNPs with identity to PT4 in every 100kb revealed that the PT4 genome under investigation had SNPs occurring between genes *rrfC* and *yhjO* (about 2/5ths of the genome) that were preponderantly similar to bf PT13a *S.* Enteritidis; however, the rest of the genome was more similar to the wt PT13a isolate. As compared to the two PT13a strains, the PT4 genomic database was genetically a dimorphic hybrid of the wt and bf PT13a isolates, which agreed with previous results obtained by pan-genomic phenotype microarray (Biolog, Inc.). Thus, the PT4 genome sequenced by the Sanger Institute exhibits a mixture of phenotypes from a single genome in response to environmental signals.

We conclude that very little genetic change is required for *Salmonella enteritidis* to alter its virulence phenotype and that the ability of bacteria to mutate rapidly obscures identification of those SNPs that are most closely linked to outbreaks of salmonellosis. Furthermore, epidemiological

investigations that are based on fingerprinting methodology are inadequate for detecting evolutionary trends due to SNPs that impact the virulence potential of the *Salmonella*. The current problem of food borne illness associated with *S. enteritidis* may have originated when a single bacterial cell was co-infected by incompatible lysogenic bacteriophage. This single cell may have rapidly split into two phage lines that nonetheless had only slightly different pathogenic potential to begin with and that overtime evolved adaptations to selection pressures present in different regions and niches within the on-farm environment.

Case Report of *Salmonella* in Poultry Meat - Questionable Labels and Confusing Products
***Salmonella enteritidis* and *Salmonella typhimurium* Outbreaks Associated with Frozen Chicken Entrees, Minnesota, 2005- 2006**

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Background

In 1998 and 2005, two outbreaks of salmonellosis associated with eating frozen, pre-browned, single-serving, microwavable stuffed chicken products were identified in Minnesota. Thirty-three cases of *Salmonella typhimurium* infection associated with Maple Leaf Farms Chicken Kiev were identified in the 1998 outbreak. Four cases of *S. heidelberg* infection associated with Cub Foods Chicken Broccoli and Cheese were identified in the 2005 outbreak. The investigations of these two outbreaks lead to minor label changes of the two specific brands of stuffed chicken products.

Two additional outbreaks associated with these types of products were identified and investigated in Minnesota in 2005 and 2006. From August, 2005 through February, 2006, the Minnesota Department of Health (MDH) Public Health Laboratory identified 13 human-case isolates of *Salmonella enteritidis* that were indistinguishable by pulsed-field gel electrophoresis (PFGE); the subtype was designated SE43B18. Routine interviews of the cases revealed that they many of the cases reported eating frozen, pre-browned, single-serving, microwavable stuffed chicken products during the week before illness onset. An investigation was initiated. During the *S. enteritidis* investigation, an outbreak of *S. typhimurium* infections associated with these products was also identified.

Methods

All *Salmonella* cases reported to MDH are routinely interviewed about food consumption and other exposures as part of enteric disease surveillance in Minnesota. A case-control study was conducted to evaluate the association of illness with stuffed chicken products. All *S. enteritidis* SE43B18 identified in surveillance that were interviewed from August, 2005 through February 2006 were included as cases. *Salmonella* cases of serotypes other than Enteritidis identified in the same time frame were used as controls. Three controls were included per case.

The Minnesota Department of Agriculture (MDA), the Centers for Disease Control and Prevention, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) and other states were notified of the *S. enteritidis* outbreak on March 8, 2006.

The MDA Dairy and Food Division collected products for testing that *S. enteritidis* and *S. typhimurium* cases had purchased at the same time as the products consumed in the week before their illness. Intact products from the same stores or chains where the cases shopped were also collected for testing. The MDA Microbiology Laboratory cultured the products for *Salmonella*, and all isolates were sent to the MDH Public Health Laboratory for PFGE subtyping.

Results

<u>*S. enteritidis* Outbreak:</u>

Eleven cases and 33 controls were included in the case-control study. Eating stuffed chicken products was statistically associated with illness (9 of 11 cases vs. 0 of 32 controls; odds ratio, undefined; 95% confidence interval, undefined; $p < 0.001$). No other exposure was statistically associated with illness.

Twenty-seven *S. enteritidis* cases with isolates of the outbreak subtype or one band different from the outbreak subtype that reported eating stuffed chicken products in the week prior to illness were identified. Dates of illness onset ranged from August 21, 2005 through July 27, 2006. Six cases were hospitalized for their infection. The median age of the cases was 31 years (range, 5 to 85 years). Unlike the two previous outbreaks, cases reported eating different flavors (Kiev, Cordon Bleu, and Shrimp and Crab) of product representing several different brands and manufacturers. Eight different brands

produced by three different manufacturers were reportedly consumed by cases. Products produced by Serenade Foods (USDA plant 2375) were reported by at least 11 cases, Aspen (USDA plant 1358) by at least five cases, and Barber Foods (USDA plant 273) by at least one case.

S. enteritidis was isolated from stuffed chicken products from three cases' households. All three were Maple Leaf Farms (USDA plant 2375) products, with production codes of S5307 and S5308, which represent production dates of November 3 and 4, 2005. No other brands were available for testing from cases' households.

Cooking methods were ascertained for 27 cases; of these, 70% cooked the products in the microwave, and one case cooked the product in a toaster oven. None of the cases took the internal temperature after cooking.

Fourteen additional *S. enteritidis* cases associated with these products were identified in nine other states.

S. typhimurium Outbreak:

Three cases with *S. typhimurium* isolates of an indistinguishable PFGE subtype reported eating Cub Foods (produced by Aspen Foods, P-1358) stuffed chicken products in the week prior to onset of illness. Dates of illness onset ranged from April 16 through June 25, 2006. Two of the cases were hospitalized. The case reported eating a variety of flavors: Kiev, Broccoli and Cheese, Mushroom and Cheddar, Mushroom and Wine, and/or Romanov. *S. typhimurium* that matched the cases' isolates' PFGE subtype was isolated from a product one of the cases purchased at the same time as the products he consumed before his illness. The product was Chicken Mushroom in Wine sauce, with a production code 5154, which represents the June 3, 2005 production date. All three cases cooked the chicken products in the microwave, and none took an internal temperature after cooking.

Public Health Interventions:

Responding to the isolation of *S. enteritidis* of the outbreak subtype, Maple Leaf Farms issued a recall on March 10, 2006. Only Chicken Broccoli and Cheese and Shrimp and Crab sold under the Maple Leaf Farms and Kirkwood labels with production codes 5307 and 5308 were recalled. In addition to the recall, on March 2006, USDA FSIS sent a letter to all processing plants that make these or similar products to those recalled, instructing them to re-evaluate the adequacy of the package labels to ensure that the consumer is aware that these products are "uncooked." Also in response to the outbreak, the National Advisory Committee for the Microbiological Criteria for Foods (NACMCF) issued new guidelines for labeling this type of product; these guidelines included: Advising consumers that microwaving raw poultry from a frozen state is not advisable unless the manufacture instructions ensures that they achieve the recommended (165ºF) endpoint temperature; the principal display panel of the label should have a warning declaration explicitly stating that the product contains raw poultry; and reminding consumers to fully cook the product when the product is raw, but gives the appearance of being fully cooked. The processing plants were required to submit the new labels for USDA approval within 8 months.

Due to the ongoing nature of the outbreak after the recall, and the recognition of the *S. typhimurium* outbreak, the Food Safety and Inspection Service, USDA issued a consumer alert on July 3, 2006. The consumer alert included instructions to consumers on needing to "take multiple temperature readings using a food thermometer at different locations throughout the product due to the non-uniformity of the heating process and the creation of "cold spots" when cooking these products in the microwave." This alert was not run in local newspapers, and did not appear to have an effect on the outbreak. On July 20, the Minnesota Departments of Agriculture and Health issued a joint press release notifying Minnesota consumers about the outbreak, and strongly advising against cooking these types of products in the microwave.

Discussion

These were the third and fourth outbreaks of *Salmonella* infections in Minnesota associated with eating frozen, pre-browned, single-serving, microwavable stuffed chicken products. Even though these

products are raw, the products' cooked appearance, and the label's microwave instructions, has lead to consumers undercooking the products. Most cases cooked the products in the microwave without thawing it first (per instructions on the labels). Despite instruction on the label to take an internal temperature to assure that these products were cooked thoroughly, none of the 30 cases took the internal temperature. The 67 cases associated with the four outbreaks associated with these products since 1998, clearly establish that these products are not safe to consumers. Under the new label requirements, consumers will more easily identify the product as raw. The producers were required to verify that the cooking instructions (time and temperature) on the label are sufficient to reach the appropriate internal temperature. However, microwave cooking instructions will still be allowed on the new labels. In order to prevent future outbreaks, we feel that microwave instructions should be removed entirely from the label, that these products are fully cooked prior to sale, or that these products are irradiated prior to sale.

Prevalence and Antimicrobial Susceptibility of *Salmonella* from Swine Operations in Five States

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Food borne illness and antimicrobial susceptibility of food borne pathogens is a growing global concern. *Salmonella* is estimated to account for a major proportion of these illnesses. To better control food borne illness due to *Salmonella* and antimicrobial resistance requires a better understanding of the ecology of *Salmonella* on the farm.

To determine the prevalence of food borne pathogens in fecal samples collected from finisher pigs on farms in five states and to characterize their susceptibility to a panel of antimicrobial drugs, fecal samples were collected and cultured quarterly from late finisher pigs on farms over a two-year period. All *Salmonella* isolates were evaluated for susceptibility to a panel of antimicrobial drugs using a micro-broth dilution system.

Overall, *Salmonellae* were recovered from 9.0% (720/7960) of the samples tested. *Salmonella* was recovered from fecal samples on 70.4% (38/54) of swine operations studied. The most common serotype of *Salmonella* recovered was *S. derby* (43.4%) followed by *S. typhimurium* 5- (16.9%) and *S. heidelberg* (10.3%). All other serotypes (n=15 plus the untypable category) comprised less than 10.0% of the *Salmonella* isolates available for serotyping. Tetracycline resistance was widespread (91.3%) among the *Salmonella* isolates while resistance to other antimicrobials was much less common. Management variables, including antimicrobial exposures, are being evaluated for association with resistance outcomes. More work is required to identify the potential risk factors related to the prevalence and antimicrobial susceptibility of food borne pathogens on United States swine operations. Such work may help to form the basis for risk mitigation strategies on the farm.