REPORT OF THE COMMITTEE ON SALMONELLA

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The Committee met on November 14th, 2010 at the Minneapolis Hilton Hotel in Minneapolis, Minn., from 12:30 p.m. until 5:45 p.m. There were 18 members and 30 guests present. After the Chair welcomed the attendees to the meeting, he reminded those present to sign the attendance sheets and note whether they might like to become a committee member; only USAHA members in good standing may join and vote in committee matters. Dr. McDonough thanked Dr. Paula Fedora-Cray for moderating last year's Committee meeting in San Diego, California while he was on sabbatical leave in Dublin, Ireland. Members were also encouraged to review the Report of the 2009 Committee meeting found on the website at http://www.usaha.org/committees/reports/2009/report-sal-2009.pdf. It was also noted that Dr. McDonough had just finished his 5 year term as Committee Chairperson and that volunteers from the Committee were needed for both a new Chair and Vice Chair.

CDC Update on *Salmonella* in the United States

LT Linda Capewell VMD, MPH, Epidemic Intelligence Service Officer, Waterborne Disease Prevention Branch, Division of Foodborne, Waterborne and Environmental Diseases, U.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA, gave an overview of *Salmonella* in the United States, updated surveillance activities of FoodNet, National Antibiotic Resistance Monitoring System (NARMS), and the National OutbreakNORS and finally covered the *Salmonella* outbreaks for the past year.

There are more than 2500 serotypes of *Salmonella*. Each year in the US, *Salmonella* infections cause an estimated 1.4 million illnesses, 168,000 physician office visits, 15,000 hospitalizations and 400 deaths. The first surveillance system presented is the Foodborne Diseases Active Surveillance Network or FoodNet. FoodNet was established in 1996 and is the principal foodborne disease component of CDC's Emerging Infections Program. FoodNet is a collaborative project of the CDC, the US Department of Agriculture, the US Food and Drug Administration and 10 participating state health departments. The FoodNet catchment area accounts for 45 million persons or approximately 15% of the U.S. population. FoodNet conducts active laboratory-based surveillance at more than 650 clinical laboratories serving the catchment area to ascertain all laboratory-confirmed infections due to seven bacterial foodborne pathogens including *Salmonella*. Enhanced surveillance of foodborne infections as measured in FoodNet sites estimates that the rate of *Salmonella* has changed the least compared to the 1996 to 1998 baseline period versus other common foodborne bacterial infections. There was a 4% decrease in the incidence of *Salmonella* in 2009 compared with the previous 3 years, but this change was not statistically significant. However, compared with the 1996-1998 period, there was a 10% decrease in *Salmonella* with a confidence interval of a 3% to 16% decrease. The healthy people objective for 2010 is 6.8 cases of *Salmonella* per 100,000 persons. The level for 2009 was 15.2 cases/100,000. This is still well above the healthy people objective of 6.8 and is furthest away from the target compared to other common foodborne bacterial pathogens. The top 10 *Salmonella* serotypes from humans in 2009 accounted for 73% of all *Salmonella* infections. *Enteritidis* and *Typhimurium* were the top 2 most common serotypes.

The next surveillance discussed was NARMS. NARMS started in 14 sites in 1996 and is a surveillance system that monitors changes in antimicrobial drug susceptibilities of selected enteric bacterial organisms in humans, animals, and retail meats among a panel of antimicrobial drugs important

in human and animal medicine. It shows trends in multidrug-resistant *Salmonella* and resistance to clinically important drugs. The NARMS program consists of three arms: the Human Arm reported through CDC, the retail arm reported through FDA-Center for Veterinary Medicine and the Animal Arm reported through USDA. NARMS expanded nationwide in 2003.

The following changes to NARMS analysis have been made. For ceftriaxone, the breakpoint for resistance changed this year from ≥ 64 µg/ml to ≥ 4 µg/ml. The revised breakpoints were applied in the 2008 report. In the 2009 report, ceftiofur was replaced with ceftriaxone resistance in the MDR-AmpC definition. Next, is an update on antimicrobial resistance among *Salmonella* isolates in 2008. 9.4% of nontyphoidal *Salmonella* isolates were resistant to greater than or equal to 3 antimicrobial drug classes. 11.5% of *Salmonella* Newport isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, amoxicillin-clavulanic acid, and ceftiofur. 22.9% of *Salmonella Typhimurium* isolates were resistant to the ACSSuT-type serovar *Typhimurium* DT104.

Next described is the role of CDC's OutbreakNet response team. This team supports a national network of epidemiologists and other public health officials who investigate outbreaks of foodborne, waterborne, and other enteric illnesses in the United States. It is a collaboration between CDC and U.S. State and local health departments, U.S. Department of Agriculture (USDA), U.S. Food and Drug Administration (FDA), and works in close partnership with PulseNet, the national molecular subtyping network for foodborne disease surveillance. From information reported on the average number of clusters the outbreak net team followed by month and pathogen from February 2008 to April 2010, *Salmonella* was the most frequent pathogen under surveillance. According to data from FoodNet, 4.45% of all *Salmonella* cases were related to outbreaks in 2009, which is a decrease compared to 7.15% in 2006, 6.09% in 2007, and 7.35% in 2008.

Next described is the National Outbreak Reporting System, or NORS, which was launched last year. It is an electronic reporting system for foodborne and waterborne disease outbreaks, enteric 'person-to-person'-transmitted disease outbreaks, and animal contact associated enteric disease outbreaks. This is a web-based system that provides one online location for reporting these types of outbreaks. Data from this system can be used for future analyses to provide more information about risk factors associated with these types of outbreaks. Additionally, it will allow for continued reporting of animal contact associated outbreaks including those associated with animals in public settings. Information provided on the number of Salmonellosis outbreaks reported to CDC from 2006 to 2010 highlights 17 outbreaks; 8 of these outbreaks were ingredient driven.

Three large multi-state outbreaks of *Salmonella* occurred within the past year and were coordinated by CDC. They involved live poultry, imported pepper and most recently shell eggs. The first outbreak was *Salmonella Typhimurium* infection associated with exposure to baby poultry in Pennsylvania in August 2009. In May 2009, the Pennsylvania Department of Health detected an outbreak of *Salmonella Typhimurium* infections with an indistinguishable pulsed-field gel electrophoresis (or PFGE) pattern in the Northeastern region of the state and was posted to PulseNet. Pennsylvania Department of Health epidemiologists conducted initial patient interviews for hypothesis generation identified exposure to live poultry as most likely source of outbreak. Additionally, some patients reported purchasing these birds at agricultural feed stores. Based on these findings, Pennsylvania requested CDC's assistance with the investigation on July 31, 2009. There were a total of 36 cases meeting the case definition; Pennsylvania had 16 cases and New York had 20 cases. Cases were clustered from May through June with a peak in late May and an additional case in august. The demographic characteristics for those that were infected with the outbreak strain showed the median age was 8 years and 31% were less than 1 to 3 years old. A case-control study was conducted and live poultry including chicks and ducklings; as well as a national feed store chain was significantly associated with illness. When cases were asked questions about the types and places of exposure they had with baby poultry, 84% touched or held birds, 21% kissed birds or put birds near their mouths, 53% were exposed to birds at home, and 47% were exposed at a feed store. There were 13 case patients that owned baby poultry either through purchase or as a gift. 92% of them bought their birds from an agricultural feed store. 85% bought from a single feed store chain and 15% received birds as gifts from relatives. States and CDC conducted tracebacks to identify the sources of these baby poultry. CDC then notified USDA-NPIP of the investigation in August 2009. NPIP subsequently led the environmental investigation to identify source flocks to the mail-order hatchery and continues to work with the hatchery on this endeavor. Most of the outbreak- associated birds were purchased from a single feed store chain. In conclusion, this outbreak was associated with exposure to live baby poultry and a single feed store chain supplied by one hatchery. Live poultry-associated human

salmonellosis is an important public health problem, and mail-order hatcheries are repeatedly implicated in these outbreaks.

The second large multi-state outbreak was *Salmonella* Montevideo infection associated with salami products made with contaminated imported black and red pepper in November 2009. Open-ended interviews identified Italian-style meats including salami as a leading hypothesis. In January 2010, the Washington State Department of Health collected shopper card information from ill persons who shopped at one warehouse store chain. They reported that 5 of 7 ill persons purchased a package of Italian Style Deli Meats from a single company and all had purchased it before their illness onset. A case-control was also conducted and results showed that case-patients were significantly more likely than controls to have eaten any salami in the 7 days before illness began, with a matched odds ratio of 8.0. Any Italian-Style meat was also significant, with a matched odds ratio of 4.5. Tracebacks revealed products from a single company were produced in three Rhode Island establishments. USDA and the Rhode Island Department of Health began an investigation of this company in January. The outbreak strain was identified from eight separate salami products. Six were open products collected from case patient households and two were intact, sealed products purchased at retail. The salami products contained a pepper coating that was applied after the lethality step of meat production. The company used both red and black peppercorn in various forms in their products. FDA conducted tracebacks to investigate pepper contamination and found that this company had three suppliers of pepper spices that originated from 3 different Asian countries. Samples of black and red pepper collected by FDA at this company tested positive for the outbreak strain. As a result of positive pepper samples from this company, specific lots of black and red pepper were recalled by two different spice companies. There were 252 persons infected with the outbreak strain from 44 states and the District of Columbia, 26% were hospitalized. There were three voluntary recalls issued by the company implicated, totaling more than 1.3 million pounds of product. The first pepper recall was issued on February 25th and to date there have been 8 other pepper recalls. In conclusion, a nationwide outbreak of *Salmonella* Montevideo infections was caused by salami products containing contaminated black and red pepper which emphasizes the potential for pepper and other spices to contaminate ready-to-eat products. Open-ended interviews, shopper card information, and rapid tracebacks were critical to the investigation.

The third outbreak was a multistate outbreak of *Salmonella Enteritidis* infections associated with shell eggs in August 2010. In July 2010, CDC PulseNet identified a nationwide sustained increase in the number of *Salmonella Enteritidis* isolates matching the outbreak strain. The PFGE pattern matching the outbreak strain is the most common PFGE pattern for *Salmonella Enteritidis* in the PulseNet database with 40 to 50 cases reported weekly to CDC. Because of the large number of expected cases, standard methods of molecular subtyping alone were not sufficient to determine which reported cases might be outbreak-associated. The number of reports increased substantially in July when the peak of the outbreak appeared to have occurred. From May 1 to October 15, 2010, a total of 3,182 illnesses were reported. Based on the previous 5 years of reports to PulseNet, 1,369 total illnesses would be expected during this same period. Therefore, 1,813 reported illnesses are likely to be associated with this outbreak. The epidemiologic approach was to focus on restaurants or event clusters where more than one ill person with the outbreak strain had eaten. Epidemiologic investigations conducted by public health officials in 11 states since April have identified 29 such restaurants or events. Wright County Egg, in Galt, Iowa, was an egg supplier in 15 of these 29 restaurants or event clusters. Traceback investigations have been completed for several of these clusters. A formal traceback was conducted by state partners in California, Colorado, and Minnesota, in collaboration with FDA and CDC, to find a common source of shell eggs. Wright County Egg in Iowa was found as the common source of the shell eggs associated with three of the clusters. Through traceback and FDA investigational findings, Hillandale Farms of Iowa, Inc., was identified as another potential source of contaminated shell eggs contributing to this outbreak. Conclusions about this outbreak revealed that Wright County Egg and Hillandale Farms of Iowa were the likely sources of the contaminated shell eggs. FDA has not found that the feed was distributed to any companies other than Wright County Egg and Hillandale Farms of Iowa.

A Multistate Outbreak of Human *Salmonella Typhimurium* Infections Associated with Aquatic Frogs–United States, 2009-

LT Linda Capewell VMD, MPH, Epidemic Intelligence Service Officer, Waterborne Disease Prevention Branch, Division of Foodborne, Waterborne and Environmental Diseases, U.S. Centers for

Disease Control and Prevention, Atlanta, GA also gave the next report on *Salmonella* infections associated with aquatic frogs.
http://www.cdc.gov/Features/salmonellafrogturtle/
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5851a1.htm

Background: *Salmonella* causes approximately 1.4 million infections annually in the United States. Although amphibians are known *Salmonella* carriers, no multistate outbreak associated with amphibians has been previously reported. During fall 2009, we investigated a multistate outbreak of *Salmonella Typhimurium* infections predominantly among children.

Methods: We conducted a matched case-control study. Cases were defined as *Salmonella Typhimurium* infection in a person whose isolate matched the outbreak strain by pulsed-field gel electrophoresis and multiple-locus variable-number tandem repeat analysis. Controls were persons with recent infection with *Salmonella* strains other than the outbreak strain and matched by age and county of residence. Environmental samples were obtained from patients' homes with subsequent tracebacks on positive samples.

Results: We identified 113 cases from 31 states with illness onset 4/1/2009 – 3/31/2010; 35% (18/54) were hospitalized and none died. Median age was five years (range = <1-73 years); 77% were <10 years. Among 18 cases and 29 controls, illness was significantly associated with exposure to frogs (67% cases vs 3% controls, mOR=24.4, CI=4.0-infinity). Among 6 case patients who knew the frog type, all reported the African Dwarf Frog (ADF), a type of aquatic frog. Environmental samples from aquariums containing ADFs in 4 patients' homes yielded isolates matching the outbreak strain. Traceback investigations of ADF's converged to a common breeder. Environmental samples from the breeder's facility yielded the outbreak strain.

Conclusions: Our investigation identified ADFs as the source of this pediatric predominant outbreak. Public education regarding risk for salmonellosis should be expanded to include risk for salmonellosis from frogs and other amphibians.

Strengthening Policy and Collaboration in Pre-harvest Food Safety-

John W. Linville, DVM, MPH, CPH, Senior *Salmonella* Pathogen Lead, Office of Policy and Program Development, Food Safety and Inspection Service, U.S. Department of Agriculture, Omaha, Nebraska (weather related complications prevented Dr. Linville from presenting his talk and Dr. William James from FSIS graciously presented the talk).

The President's Food Safety Working Group (FSWG) established core principles to help Food Safety Agencies like FSIS target areas in the farm-to-table continuum where more attention is needed. One area within this continuum that FSIS is focusing on is strengthening policies around pre-harvest controls for *Salmonella*.

FSIS actively provides an array of verification and monitoring sampling results to regulated establishments, such as qualitative (positive or negative) *Salmonella* verification sample results. FSIS intends to increase the value of this information by enhancing it with additional data, such as by providing detailed subtype (serotype and PFGE pattern) and antimicrobial susceptibility data on positive samples, as well as quantitative information, when available. FSIS believes that by sharing such additional information with establishments, this action will make them better aware of pathogens on their products so that they can consider actions to reduce future food safety hazards on products more proactively. FSIS expects that establishments will, in turn, share this information with the individual producers so that the producers can take steps to prevent, eliminate, or reduce to an acceptable level the FSIS-identified food safety hazard in subsequent shipments of animals and egg products to FSIS for inspection.

In addition, FSIS has collaborated with the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) through the FSWG to develop a set of metrics specifically for *Salmonella Enteritidis* (SE). FSIS is looking at controls, such as those recently required by FDA for egg laying flocks, as well as controls required in the European Union (EU), to develop policies that will actively support reducing human foodborne salmonellosis caused by SE in broilers, as well as egg products. SE exposure associated with broilers has been increasing in recent years and is a rising public health concern. Finally, FSIS is considering a wide variety of collaborative strategies and policy options to encourage establishments to strengthen the pre-harvest area of their food safety systems. The Agency is seeking input from the committee on these collaborative strategies.

NVSL *Salmonella* Update

The annual update from the National Veterinary Services Laboratories was provided by Matt Erdman, DVM, PhD, Head-Bacterial Identification, Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, USDA-APHIS-VS, Ames, IA. Dr. Erdman noted that NVSL has added multiple-locus variable-number tandem repeat analysis (MLVA) to their battery of *Salmonella* subtyping tests, antimicrobial susceptibility testing (TREK Sensititre system), also new *Salmonella* panels, a new federal 10-3 submission form for *Salmonella* serotyping requests, and also a new laboratory reporting system.

They also added a *Salmonella Enteritidis* (SE) or "SE Rule Out Test" in July 2010 to assist with the FDA Egg Rule. The purpose of this test was to rapidly identify or confirm a Group D *Salmonella* isolate as SE or not SE. The results will be available within 1-2 business days after receipt of the isolate.

NVSL also offers a *Salmonella* Group D Proficiency Test which tests the ability to isolate *Salmonella*, if present, and to further identify Group D if present. Results of this testing are to be found in the Serotype addendum at the end of this Report. The next *Salmonella* Group D Proficiency Test will be offered in the spring of 2011.

Chicken submissions, both clinical and non-clinical, included 1089 Group D *Salmonella* isolates; of these 993 (92%) were SE and the other 8% of the serotyped comprised *S. Berta, S. Alabama, S. Dublin, S. Javiana, S. Ouakam*, and *Salmonella* 9,12:nonmotile. It is noteworthy that no *S. Pullorum* was found in this group of isolates. Of the SE isolates submitted to NVSL the following Phage Types were found in decreasing order of frequency Phage type 8, 13a, 23, 13, and "other."

As far as molecular typing of *Salmonella* at NVSL, technologies have been evaluated and a Luminex-based assay developed by CDC has been implemented. NVSL will continue to test submitted isolates by both conventional serotyping and molecular typing methods, and will maintain the ability to perform the gold standard of conventional serotyping.

The complete text of the *Salmonella* serotyping presentation is included at the end of this report as an addendum.

NARMS and VetNet Updates-

Paula J. Fedorka-Cray, PhD, Research Leader, USDA-ARS-Bacterial Epidemiology and Antimicrobial Resistance (BEAR), Athens, GA gave her yearly overview and summary of the activities of her research team including the animal NARMS data and VetNet updates:
http://www.ars.usda.gov/Main/docs.htm?docid=6750
http://www.ncbi.nlm.nih.gov/pubmed/17600492
http://www.aphl.org/profdev/conferences/proceedings/Documents/2005_9th_Annual_PulseNet_Update_Meeting/Cray_USDA_VetNet.pdf

The first item of business was an update of the animal arm of the National Antimicrobial Resistance Monitory System (NARMS). Diagnostic isolates included in animal NARMS are presumed to be associated with clinical illness in the host animal. These isolates are from hosts not likely to enter a slaughter facility. Isolates from sentinel sites (14 veterinary diagnostic labs) stopped in 2006 due to lack of funding for this effort. However, a random selection of clinical isolates from the National Veterinary Services Laboratories are used in this effort and in the past Sentinel states would have been excluded from NVSL selection to prevent duplication. Non-diagnostic isolates used in animal NARMS are presumed to come from healthy animals and include on-farm and slaughter sources. On-farm isolates have come from National Animal Health Monitory System (NAHMS) studies of national prevalence which includes a 5 year rotations of the commodity; slaughter isolates include rinsates, carcass swabs, ground product, ready-to-eat (RTE) foods, and eggs. Slaughter isolates are thought to provide a comprehensive snapshot of what is going to the retail arena from compliance testing.

Dr. Cray reviewed the trend of pan-susceptible *Salmonella* isolates human versus animal (by species); the resistance of *S. Newport's* from human versus animals (to these drugs: to Ampicillin, Chloramphenicol, Streptomycin, Sulfanomides, Tetracycline, Amoxicillin-Clavulanic Acid, and Ceftiofur). Also presented were the percent of *S.Typhimurium* (including *Typhimurium* var 5-) isolates resistant to at least Ampicillin, Chloramphenicol, Streptomycin, Sulfanomides, Tetracycline (ACSSuT) and the percent of resistant confirmed *S.Typhimurium* DT104 (1997-2009) all of which were Slaughter isolates versus human trends. The distribution of DT104 isolates from Slaughter (cattle, chicken, swine, turkey) from 1997-2009 continues to decline. The trends in multiple drug resistance (>= 5 drug classes) among *S.Enteritidis, S.Heidelberg, S. Kentucky, S. Typhimurium and S. Typhmurium* var 5- were presented. The overall resistance patterns from both cattle and chickens were presented for the time period 1997 to 2009.

A review of VetNet was presented. Foodborne pathogens: non-typhoidal *Salmonella* are analyzed with *Campylobacter* being added in 2005. A dedicated server houses all USDA VetNet PFGE patterns and is located in GA. The VetNet group is currently only analyzing gels sent by USDA-ARS group in Athens, GA. The VetNet *Salmonella* Database was started in May 2004 and contains isolates from slaughter, diagnostic, and on-farm sources. The isolates are analyzed primarily with 1 enzyme cuts, and all isolates are assigned a VetNet pattern name. Starting with the top 30 serotypes of the Public Health Laboratory Information System (PHLIS) the VetNet (VN) patterns are compared to PulseNet (PN) patterns, if a match occurs, both patterns are listed in the database. As of As of July 21, 2010 the VetNet database contained 19,184 isolates, with 4,792 unique XbaI patterns from 267 *Salmonella* serotypes. The USDA's VetNet program continues to communicate with the CDC, e.g., the *S.Enteritidis* PFGE pattern JEGX01.0004 found by CDC from an outbreak in humans was found to match *S.Enteritidis* PFGE pattern JEGX01.0061 from VetNet; JEGX01.0061 was a new pattern in VetNet as of 04-20-10 and was detected from 7 isolates from chicken carcass rinses.

A temporal series of PFGE Profiles for different *Salmonella* serotypes were presented including their antimicrobial resistance patterns, e.g., *S.Enteritidis*, *S. Anatum*. The data comprise primarily one enzyme cuts, and starting in 2011 will include a 2nd enzyme cut. There is concern for the definition of 'fingerprint' and what is a 'match' between isolates? It is useful to understand that band differences can be attributed to genetic changes, plasmids, etc. Most isolates require additional info for analysis such as antimicrobial resistance information, plasmid or other genetic information; supporting epidemiology including the context of the isolate in important plus the methodology used in the analysis of the isolate. VetNet is now accepting Tiff files and/or isolates from animal sources for inclusion in VetNet; however, gel certification required to send Tiff files.

Upcoming changes to NARMS and to VetNet include the establishing of NARMS as a separate CRIS project; the redesign of the sampling scheme for NARMS by adding sampling on the farm with Swine as the first commodity to be studied. The top 3 states will be Iowa, Minnesota and North Carolina. Poultry will be next to be studied on farm. NARMS will be adding methicillin Staphylococcus aureus (MRSA) and Clostridium difficile. USDA will be expanding VetNet to include a website.

Evolutionary Trends and Combinatorial Complexity of *Salmonella Enteritidis*-

Jean Guard, DVM, PhD**,** Egg Safety and Quality**,** Veterinary Medical Officer**,** USDA, ARS, SAA**,** ESQRU, Athens, GA, presented an ARS research update from her team's work on the molecular biology of *Salmonella Enteritidis*.

http://www.ncbi.nlm.nih.gov/genomes/static/Salmonella_SNPS.htm

Salmonella enterica serovar *Enteritidis* (*S.Enteritidis*) is currently the world's leading cause of salmonellosis, in part because of its ability to contaminate the internal contents of eggs produced by otherwise healthy hens. High-density tiling analysis of two PT13a strains that vary in the ability to contaminate eggs and from other genomic studies indicate that *S.Enteritidis* evolution is driven by variant patterns of single nucleotide polymorphisms (SNPs) that most often escape detection by commonly used epidemiological methods. To date, 247 sequence-confirmed SNPs on the chromosome and external to lysogenized bacteriophage have been linked to phenotypes that vary in virulence potential. Patterns of mutation suggest that adaptive radiation rather than randomly occurring genetic drift is driving evolution of *S.Enteritidis*. The combinatorial complexity present in circulating strains of *S.Enteritidis* is evident, but progress is being made on incorporating assays for detection of virulent subpopulations into serotyping schema. Genomic analyses require stringent application of biostatistics and biological studies to meet the objective of reducing *S.Enteritidis* in the food supply.

Subpopulation biology occurring within and between serotypes of *Salmonella* enterica may be used one day to: implement effective competitive exclusion in mature flocks as well as in chicks; to improve vaccination strategies; and to raise flock immunity to more deleterious strains; and lastly to impede the environmental presence and invasive infections of SE on-farm.

National Poultry Improvement Plan's (NPIP) Status Report-

C. Stephen Roney, DVM,MAM, Veterinary Coordinator, National Poultry Improvement Plan, USDA, APHIS, VS, Conyers, GA gave an update of the NPIP program.

The complete text of the *Salmonella* serotyping presentation is included at the end of this report as an addendum.

2010 Outbreak: FDA's response to *Salmonella Enteritidis* in shell eggs-

Tracy S. DuVernoy, DVM, MPH, DACVPM, Veterinary Medical Officer, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Defense, Communication and Emergency Response, Emergency Coordination and Response Team, College Park, MD presented a review and status update to the national outbreak of SE linked to shell eggs form Iowa flocks. Her report included an overview of the outbreak, the timeline of initial incident, the FDA investigation, and then preventive controls, laboratory findings, the status of the current Situation, and then conclusions.
http://www.fda.gov/NewsEvents/Testimony/ucm226554.htm
http://www.fda.gov/Food/NewsEvents/WhatsNewinFood/ucm222684.htm
http://www.cdc.gov/salmonella/enteritidis/se_timeline_092010.pdf

In July 2010, CDC identified a nationwide sustained increase in the number of *Salmonella Enteritidis* isolates with PFGE pattern JEGX01.0004 was uploaded to PulseNet. This increase began in May 2010 and is evident in the epidemic curve. The number of reports increased substantially in July when the peak of the outbreak appears to have occurred. From May 1 to September 14, 2010, a total of 2,752 illnesses were reported. However, some cases from this period have not been reported yet, and some of these cases may not be related to this outbreak. Based on the previous 5 years of reports to PulseNet, we would expect approximately 1,144 total illnesses during this same period. This means there are approximately 1,608 reported illnesses that are likely to be associated with this outbreak. Many states have reported increases of this pattern since May. Because of the large number of expected cases during this period, standard methods of molecular subtyping alone are not sufficient to determine which reported cases might be outbreak-associated. CDC is currently evaluating advanced molecular methodologies to see if they help distinguish between outbreak-related cases and sporadic (or background) cases. Illnesses that occurred after August 12, 2010 might not yet be reported due to the time it takes between when a person becomes ill and when the illness is reported. This typically takes two to three weeks for *Salmonella*, but can take up to six weeks. A formal traceback was conducted by state partners in California, Colorado, and Minnesota, in collaboration with FDA and CDC, to find a common source of shell eggs. Wright County Egg in Iowa was found as the common source of the shell eggs associated with three of the clusters. The Incident Management Group was mobilized from August 10, 2010 through September 3, 2010. However, FDA continued to work and take immediate action to prevent imminent harm to public health from contaminated shell eggs and products derived from eggs through normal agency operations. Through traceback and FDA investigational findings, Hillandale Farms of Iowa, Inc., was identified as another potential source of contaminated shell eggs contributing to this outbreak.

Short term preventive controls included the diversion of shell eggs to an official USDA, FSIS approved breaker facility for pasteurization occurred and on August 13, 2010, Wright County Egg of Galt, Iowa, conducted a nationwide voluntary recall of eggs which was expanded on August 18, 2010. On August 20, 2010, Hillandale Farms of Iowa conducted a nationwide voluntary recall of shell eggs. The brands listed were either recalled by these two firms or were recalled by other firms who received the eggs and repacked them under additional brand names. The eggs were distributed in a variety of sizes and packaging configurations. An egg noodle recall was issued on Sept. 3, 2010: Real Taste Noodle Manufacture of Chicago, IL recalled bags of Egg Noodle because of the potential to be contaminated with *Salmonella;* bags were distributed between 06/12/2010 and 08/25/2010 to restaurants and grocery wholesalers. As of today, no illnesses have been reported to the manufacture. This recall has been initiated due to recent massive egg recall by egg-producing companies. Eggs that the manufacturer used in the manufacture of egg noodles from June to August, 2010 could be contaminated with *Salmonella*. With the assistance of State agencies, over 2100 recall audit checks were performed and were deemed effective.

Environmental assessments of premises then included the sampling of layer farms, sampling at feed mill, sampling at renderers, and monitor compliance with the FDA Egg Safety Rule (21 CFR 118). Over 600 samples collected as part of investigation, and of these 13 samples were a PFGE match to the outbreak strain, i.e., from the Wright County Egg samples: 4 positive environmental samples matched the DNA fingerprint of the outbreak strain of *Salmonella Enteritidis* (from farm #2 and farm #4). These were swab samples collected from manure, as well as traffic areas such as walkways, equipment, and other surfaces in and around the facility. Five positive samples collected from the feed mill included 1 finished feed (for pullets); 1 Meat and Bone Meal; and 3 environmentals. The finished feed was provided to pullets raised at Wright County Egg facilities in Iowa. Pullets are distributed to all premises at Wright County Egg in Iowa and illandale Farms in Iowa. From Hillandale Farms positive samples included an egg wash water sample, at the

Alden, IA location, and 3 swabs from West Union, IA location. Based on laboratory information and the FDA investigation, Wright County Egg and Hillandale Farms were deemed the likely source of SE contaminated shell eggs that caused the nationwide outbreak.

From the FDA investigation, Form FDA 483 (which is issued when investigators observe any significant objectionable conditions or practices that indicate that an FDA-regulated product is in violation of FDA's requirements), 483 observations were cited, i.e., issued to Hillandale Farms on August 27, 2010 for failure to fully implement firm's SE prevention plan; pullet documentation failure; biosecurity breach; among the observations noted by FDA investigators: failure to fully implement and follow procedures in its *Salmonella Enteritidis* Prevention Plan. Examples: failure to eliminate entryways for rodents and other pests into the egg production facilities; failure to bait and seal rodent burrow holes in the egg production facilities and to eliminate the potential rodent or pest harborage places near the structures; failure to eliminate standing water adjacent to the manure pits or to eliminate liquid manure. Investigators observed that the company failed to maintain documentation that 19-week-old pullets were monitored for *Salmonella Enteritidis*, or raised under SE-monitored conditions. Also, failure to take steps to make sure that SE isn't transferred into or among poultry houses: investigators observed uncaged hens tracking manure from the manure pits to the caged house areas. The observations issued to Wright County Egg on August 30, 2010 were for failure to fully implement the firm's SE prevention plan, i.e., failure to fully implement and follow procedures in its *Salmonella Enteritidis* Prevention Plan. Examples include: failure to prevent stray poultry, wild birds, cats and other animals from entering poultry houses. Outside access doors to manure pits were pushed out by the weight of manure which was piled in some cases four to eight feet high thereby providing openings into the poultry houses for wildlife or other animals. Animals, including rodents, were able to enter the poultry houses due to structural damage that included things like missing siding and air vents or gaps at the bottom of doors. Failure to eliminate birds from laying houses and to control rodents or flies: investigators observed bird nests and birds in one poultry house, live rodents in at least one poultry house at several plants, and live and dead flies that were too numerous to count in poultry houses at certain plants. Live flies were observed on and around egg belts and walkways to different sections of the egg laying areas. Live flies were crushed underfoot when employees walked in the aisles at work and there were live and dead maggots observed in the manure pit at one plant. Investigators observed the failure to implement practices to protect against the introduction or transfer of *Salmonella Enteritidis* between and among poultry houses. Specifically, investigators observed a lack of separate entrances to each poultry house, thus requiring the use of shared corridors between certain houses. Employees were observed failing to change protective clothing when moving from one house to another, and failed to clean and sanitize equipment prior to moving between poultry houses at one plant.

Longer term controls include the following: over the next 15 months, Food and Drug Administration (FDA) investigators will team up with other state and local partners to visit about 600 egg producers—those with 50,000 or more laying hens—to determine if their facilities are in compliance with an egg safety rule that went into effect in July. This represents 80% of where the country's eggs are produced. Some objectives of the inspections are to inspect establishments to assess compliance with 21 CFR 118: (Prevention of *Salmonella Enteritidis* (SE) in Shell Eggs During Production, Storage and Transportation Rule) to include evaluation of the SE prevention plan, evaluation of the egg laying operation, evaluation of firm's environmental testing and appropriate actions taken if a positive sample was found, and a record review.

They will also be conducting environmental sampling and inspections at egg laying farms to determine if the firm is practicing prevention measures of *Salmonella Enteritidis* contamination of the egg and egg production areas; to conduct laboratory analyses of environmental samples; and to document inspectional and analytical findings and initiate compliance action as warranted.

Next Dr. DuVernoy reviewed the "Egg Safety Rule" or the 21 CFR 118 Prevention of *Salmonella Enteritidis* in Shell Eggs During Production, Storage, and Transportation. The Rule came into effect for large producers (those with 50,000 or more laying hens) on July 9, 2010. For producers with 3,000 to 50,000 hens the regulations will become effective on July 9, 2012. FDA believes that as many as 79,000 illnesses and 30 deaths due to consumption of eggs contaminated with the *Salmonella Enteritidis* may be avoided each year with new food safety requirements for large-scale egg producers.

The current situation in Iowa: Wright County Egg was re-inspected by the FDA in October 2010 and environmental assessments were performed to evaluate corrective actions issues earlier. Samples are still being processed, but a Warning Letter was issued on October 15, 2010 for "Failure to take prompt

corrective action may result in regulatory action"; additional regulatory actions could include, but are not limited to, seizure and/or injunction. Wright County Egg continues to divert shell eggs to the breaker plant.

As far as Hillandale Farms they are no longer producing shell eggs at the Alden, IA farm, but the FDA re-inspected the West Union premises in mid-October and found that the corrective actions were adequate.

The U.S. Food and Drug Administration issued a letter to Hillandale Farms on October 15 that authorizes the company to resume shipping eggs from three of its egg-production houses. The decision, according to federal officials, was based on a thorough review of the company's response to problems that were noted during August inspections. The three houses have also been "extensively tested," according to the government, and "found to have no evidence of *Salmonella* contamination. Four additional houses under the Hillandale Farms umbrella continue to be tested and inspected, and are not eligible at this time to begin shipping eggs to market. The company has "committed to an enhanced surveillance program for *Salmonella*," according to federal documents. One stipulation the company has agreed to is monthly environmental testing of four houses for the life of the current flock of hens.

According to CDC, this represents the largest *Salmonella Enteritidis* (SE) outbreak reported since the start of outbreak surveillance in the early 1970s. The largest previous outbreak was in 1994, due to contaminated commercial ice cream, with 743 reported cases. Potential contributing factors include the feed and feed components, the presence of insects and rodents, the hen laying environment, and the laying hens. FDA is still working with State partners and the CDC to better understand this outbreak. Additional diagnostics are ongoing. FDA is working internally on an After Action Report regarding FDA's response to this event. Since the new Egg Safety Rule came into effect on July 9, 2010 for producers with more than 50,000 birds, FDA has been working with industry to inform them of the new regulations and has issued a draft guidance in August 2010: Prevention of *Salmonella Enteritidis* in Shell Eggs During Production, Storage, and Transportation. FDA will continue its outreach sessions with producers and others around the country this fall. FDA also began inspecting all large shell egg producers to make sure they are in compliance with the Egg Safety Rule as mentioned previously.

Rapid and Cost Efficient *Salmonella Enteritidis* Testing-

Jennifer Manion, Product Manager, SDIX, Newark, DE presented information on a commercial test in development for use in meeting the laboratory aspects of the 21 CFR 118 "Egg Safety Rule".
www.sdix.com

Their test is called RapidChek® SELECT™ *Salmonella Enteritidis* as a screening test and RapidChek® CONFIRM for the confirmation test. The test is designed for testing both environmental drag swabs and egg pools and has recently received AOAC approval. The test methodology is based on the use of bacteriophage to clean up the sample matrix, monoclonal antibodies and immunomagnetic separation of *Salmonella*.

Detection of *Salmonella Enteritidis* in Eggs and Poultry Environment with Real-Time PCR-

Peyman Fatemi, Ph.D., Senior Technical Applications Specialist, Food & Environmental Testing, Applied Molecular Testing, Foster City, CA (Applied Biosystems, Life Technologies) presented information on a commercial test in development for use meeting the laboratory aspects of the 21 CFR 118 "Egg Safety Rule".
www.appliedbiosystems.com

Their test is called RT-PCR SE Assay. The test is designed for testing both environmental drag swabs and egg pools and as indicated is based on RT-PCR technology.

Committee Business-

Dr. McDonough closed the presentation by thanking all the speakers.

During the business meeting he related a request from the USAHA Committee on International Standards to review appropriate (relating to *Salmonella*) chapters from a listing of 49 OIE Terrestrial Code Chapters. Primary committees should review the proposed changes and determine if the committee should comment. If there are additional chapters that may be pertinent to your committee please feel free to review as well. The deadline for comments to USDA is December 6, therefore if you could submit any comments that your committee may have to Dr. Don Hoenig by December 1 for review and submission.

It was again noted that Dr. McDonough had just finished his 5 year term as Committee Chairperson and that volunteers from the Committee were needed for both a new Chair and Vice Chair.

Members were encouraged to read, review, and perhaps comment on the Committee on *Salmonella*'s Mission Statement as recommended by the Executive Board.

Dr. McDonough closed by stating that it is an ongoing challenge to keep a balance of species issues current before the Committee, i.e., bovine, porcine, avian, exotics, equine, and amphibian/reptile.

Among future issues that the Committee could address are FDA's concern for *Salmonella* in animal feeds, the feeding of commercial raw meat diets to companion animals, and the issues of consumption of unpasteurized milk and milk products by humans.

Salmonella serotypes isolated from animals in the United States: January 1 – December 31, 2009

M.M. Erdman, B.R. Morningstar-Shaw, D.A. Barker, T.A. Mackie, M.I. Munoz, E.A. Palmer, M.A. Kane, L.K. Cox

Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, USDA

The Diagnostic Bacteriology Laboratory within the National Veterinary Services Laboratories (NVSL) routinely serotype *Salmonella* isolates submitted by private, state, and federal laboratories as well as veterinarians, researchers and other animal health officials. Most submissions were from diagnostic laboratories across the U.S., and although only counted as a single submitter, these labs typically submitted *Salmonella* isolates from a variety of sources, herds, or flocks. This report summarizes *Salmonella* serotyping submissions to NVSL from January 1 through December 31, 2009. The *Salmonella* isolates are identified as clinical (clinical signs of salmonellosis from primary or secondary infection) or non-clinical (herd and flock monitoring programs, environmental sources, food). Serotyping data from isolates submitted for research purposes are not included in the source specific summaries. Based on information provided by the submitter the isolates were divided into animal source categories for analysis. The animal sources include Avian (avian of unknown origin, parrot, pheasant, pigeon, rhea, emu, ostrich, quail, duck, and owl), Cattle, Chicken, Dog/Cat, Horse (horse, donkey), Other Domestic (alpaca, ferret, goat, guinea pig, hamster, hedgehog, llama, mink), Pigs, Reptiles/Amphibians (iguana, lizard, reptile, snake, turtle, amphibian, frog, toad), Turkey, Wild/Zoo (antelope, bat, bear, beaver, bison, deer, elk, fish, fox, marine mammals, mongoose, opossum, rabbit, raccoon, rodent, otter, wolf, squirrel, reindeer, camel, elephant, kangaroo, monkey, primate, tapir, tiger, zebra, rhinoceros, wallaby), and Other (environment, water, feed, insects, unknown).

Salmonella serotyping at the NVSL is an ISO 17025 accredited test. Sera used for typing *Salmonella* isolates consists of polyvalent sera against the O serogroups and single factor sera against the individual O and H antigens. Approximately 50% of the sera used at the NVSL are produced in house as previously described (Ewing), and the rest is purchased from commercial vendors. All sera are subjected to quality control testing prior to use. *Salmonella* antigenic formulae are determined essentially as previously described (Ewing) and interpreted via the White-Kauffmann-Le Minor scheme (Grimont). 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 formula. 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.

In 2009 there were 15,198 submissions for *Salmonella* serotyping originating from 47 different states. Of these, 480 were identified as not *Salmonella*, contaminated, or mixed culture and were not further tested. The remaining 14,718 *Salmonella* isolates were divided into clinical isolates (5,278), non-clinical isolates (8,119) and research isolates (1,321). The sources of clinical and non-clinical *Salmonella* isolates are shown in Table 1. There were 399 different serotypes identified in 2009. Table 2 lists the 10 most common serotypes when all animal sources were combined. The most common isolates from chickens, turkeys, cattle, pigs, horses, and dog/cat are listed in Tables 3-8.

The NVSL provided a *Salmonella* proficiency test in order for laboratories to assess their ability to isolate *Salmonella* from environmental samples and determine the serogroup of any *Salmonella* isolated. The samples consisted of drag swabs spiked with *Salmonella* and/or common contaminants. The 2010 test included *Salmonella* serotypes *Enteritidis, Kentucky, Berta, Heidelberg*, *Escherichia coli*, *E. coli* (H_2S+), *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The test consisted of 5 samples which were shipped to laboratories overnight on ice packs. Laboratories were instructed to use whatever protocol they choose and to report the results within 3 weeks. The NVSL randomly retained 10% of the test kits and tested them blindly for QA purposes. The results of the proficiency test are shown in Table 9.

Table 1: Sources of submissions to the NVSL for _Salmonella_ serotyping in 2009

Source	No. Clinical Submissions	No. Non-Clinical Submissions	Total
Avian	280	124	404

Cattle	1529	339	1868
Chicken	154	4607	4761
Dog/Cat	114	4	118
Horse	862	76	938
Other	182	1619	1801
Other Domestic	85	2	87
Pig	1586	357	1943
Reptile/Amphibian	126	19	145
Turkey	198	957	1155
Wild/Zoo	162	15	177
Total	**5278**	**8119**	**13397**

Table 2: Most common serotypes in 2009: All sources

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Typhimurium var 5-	605	Kentucky	1214
Typhimurium	527	*Enteritidis*	1151
Newport	349	Heidelberg	875
Dublin	290	Senftenberg	397
Cerro	246	Montevideo	256
Derby	208	Typhimurium	250
Anatum	159	Hadar	217
Agona	158	Mbandaka	191
Heidelberg	151	Typhimurium var 5-	180
Montevideo	148	Agona	148
All others	2437	All others	3240
Total	**5278**	**Total**	**8119**

Table 3: Most common serotypes in 2009: Chickens

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Enteritidis	49	Enteritidis	944
Heidelberg	20	Kentucky	930
Kentucky	15	Heidelberg	633
Senftenberg	13	Senftenberg	180
Typhimurium	9	Mbandaka	145
All others	48	Montevideo	119
		Schwarzengrund	90
		Typhimurium	82
		Anatum	60
		Berta	59
		All others	1365
Total	**154**	**Total**	**4607**

Table 4: Most common serotypes in 2009: Turkeys

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Senftenberg	46	Senftenberg	170
Ouakam	16	Hadar	132
Montevideo	15	Worthington	107
Heidelberg	15	Muenster	61
Hadar	14	Saintpaul	48
All others	92	London	45

		Agona	35
		Albany	28
		Schwarzengrund	25
		Montevideo	21
		All others	285
Total	**198**	**Total**	**957**

Table 5: Most common serotypes in 2009: Cattle

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Dublin	278	Kentucky	81
Cerro	226	Montevideo	57
Newport	140	Dublin	42
Montevideo	95	Cerro	25
Typhimurium	94	Typhimurium	14
Typhimurium var 5-	63	Muenchen	13
Kentucky	62	Newport	13
Muenster	57	Typhimurium var 5-	11
Agona	42	I 4,5,12:i:-	10
Meleagridis	42	Meleagridis	8
All others	430	All others	65
Total	**1529**	**Total**	**339**

Table 6: Most common serotypes in 2009: Pigs

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Typhimurium var 5-	413	Derby	29
Derby	196	Typhimurium var 5-	22
Typhimurium	144	Infantis	16
Agona	90	Typhimurium	9
Heidelberg	80	Heidelberg	8
Infantis	65	All others	67
Anatum	48		
I 6,7:nonmotile	39		
Choleraesuis	38		
Senftenberg	36		
All others	437		
Total	**1586**	**Total**	**170**

Table 7: Most common serotypes in 2009: Horses

All Sources	
Serotype	**No. Isolates**
Javiana	177
Typhimurium	155
Newport	106
Anatum	56
Braenderup	48
I 4,5,12:i:-	26
Infantis	19
Muenchen	18
Mbandaka	17
Typhimurium var 5-	17
All others	299
Total	**938**

Table 8: Most common serotypes in 2009: Dogs and Cats

All Sources	
Serovar	**No. Isolates**
Newport	31
Ohio	8
Typhimurium	6
Typhimurium var 5-	6
Mbandaka	4
Infantis	4
Livingstone	4
Kiambu	3
Javiana	3
Anatum	3
All others	46
Total	**118**

Table 9: Summary of NVSL *Salmonella* proficiency test

	2009	**2010**
Participants	40	55
Mean Score	93%	92%
Score Range	100-44%	100-44%
Below Passing	4	3

References

Ewing, WH. 1986. Edward and Ewing's Identification of Enterobacteriaceae. 4th edition. Elsevier Science Publishing Co., Inc., New York, U.S.

Grimont, PAD, Weill, FX. 2007. Antigenic Formulae of the *Salmonella* Serovars. 9th edition. WHO Collaborating Centre for Reference and Research on *Salmonella*. Paris, France.

National Poultry Improvement Plant (NPIP)
National Plan's Status Report

Steve Roney
National Poultry Improvement Plan
USDA-APHIS-VS

Pullorum-Typhoid Status:

There were no isolations/outbreaks of *Salmonella pullorum* in 2009 nor in FY 2010. There have been no isolations of *Salmonella gallinarum* since 1987 in any type poultry.

Hatchery Participation in the National Poultry Improvement Plan Testing Year FY2010	
Egg and Meat-Type Chickens: Participating	275
Turkeys Participating	40
Waterfowl, Exhibition Poultry and Game Birds	790

Egg-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2010	
U.S. Pullorum-Typhoid Clean: Participating- Number	203
Birds in Flocks-Number	3,562,748
Average per Flock	17,550

Meat-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2010	
U.S. Pullorum-Typhoid Clean: Participating- Number	5575
Birds in Flocks-Number	83,278,808
Average per Flock	14,937

Turkey Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2010	
U.S. Pullorum-Typhoid Clean: Participating –Number	824
Birds in Flocks-Number	6,789,659
Average per Flock	8,240

Waterfowl, Exhibition Poultry, and Game Birds Breeding Flocks In the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2010	
U. S. Pullorum-Typhoid Clean Participating	2975
Birds in Flocks	1,345,462

Mycoplasma gallisepticum* , *Mycoplasma synoviae, and ***Mycoplasma meleagridis*** positive breeding flocks National Poultry Improvement Plan FY2010				
	WEGBY	Egg-Type	Meat-Type	Turkeys
Mycoplasma gallisepticum	11	2	6	0
M. synoviae	16	2	53	3
M. meleagridis		0	0	0

U.S. *Salmonella enteritidis* Clean- Egg-Type Chickens
No. of flocks and birds in flocks by State with *Salmonella enteritidis* isolates, 1990-2010

Arkansas	Environmental	Dead Germ	Bird
Flocks	1		15000
Birds in Flocks	6000		2
Georgia			
Flocks	3	2	
Birds in Flocks	30400	46000	
Illinois			
Flocks	3	2	1
Birds in Flocks	3900	3700	1200
Indiana	Environmental	Dead Germ	Bird
Flocks	15	2	1
Birds in Flocks	158345	27479	15092
Kentucky			
Flocks	1		
Birds in Flocks	6625		
Ohio			
Flocks	17		9
Birds in Flocks	192700		91600
Oregon			
Flocks	2		
Birds in Flocks	19516		
Pennsylvania			
Flocks	16		6
Birds in Flocks	166385		78450
Texas			
Flocks	1		
Birds in Flocks	10000		

Phage type13	Environmental	Dead Germ
Flocks	11	2
Birds in Flocks	152000	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	21	
Birds in Flocks	237701	

Egg-type Chicken breeding flocks with isolates of *Salmonella enteritidis* by phage type and by year 1989-2008		
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
2007	4	13, 8
2008	3	8
2009	0	
2010	3	8(2), 13

***U.S. Salmonella enteritidis* Clean - Egg-Type Chickens No. of flocks and birds in the flocks with *Salmonella enteritidis* isolates, 1990-2010**			
	Environmental	Dead Germ	Bird
Flocks	71	6	19
Birds in Flocks	706,871	77179	201,342