Report of the Committee on Salmonella
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The Committee met on October 27, 2015 at the Rhode Island Convention Center in Providence, Rhode Island, from 8:00 to 12:00pm. There were 23members and 18 guests present. After the Chair opened the meeting and welcomed the attendees, he reminded those present to sign the attendance sheets. Members of the committee should check to see that their contact information was correct and if they were not members they were to sign the blank sheets and they could indicate if they would like to become a member of the committee. The Chair briefly overviewed the requirements of becoming a member and that only members could propose resolutions, recommendations and vote. However, everyone was encouraged to participate in the discussion. There were no pending Resolutions from the previous year.

2015 Enteric Zoonoses Outbreaks: Public Health Impacts and Challenges
Megin Nichols DVM, MPH, DACVPM

Enteric Zoonoses Activity Lead
Outbreak Response and Prevention Branch
Division of Foodborne, Waterborne and Environmental Diseases
National Center for Emerging and Zoonotic Infectious Diseases
Centers for Disease Control and Prevention

The Enteric Zoonoses Activity group are investigating 3 Salmonella outbreaks, Salmonella Muenchen in crusted geckos, 2 outbreaks in small turtles, and 4 multistate outbreaks of live poultry. For the last several years there have been outbreaks due to live poultry, including this year, however there is a significant fewer number of cases this year. This reduction has been attributed to increased pressure on the hatcheries to reduce Salmonella and continued efforts and outreach from the NPIP.

Another outbreak investigation was in Washington state and involved Salmonella 4,(5),12:I:- . This Salmonella was first seen in Europe in the 1990's. Human illness in the United States due to this strain has been increasing over the last 10 years. The source of this outbreak was pork, and was traced back to one processing plant. There were actually 5 PFGE patterns seen in the outbreak strain. There was also a market and restaurant found to be environmentally positive for the outbreak strain. Also beef was contaminated from a slicer used to cut the contaminated pork.

Salmonella I 4,(5),12:i:- Cluster Associated with Pork Consumption in Washington State
Dr. Karen Becker DVM, MPH, DACVMPM

Applied Epidemiology Staff
Office of Public Health Science

Food Safety Inspection Service

FSIS assisted in the investigation of the outbreak in Washington. They collected cecal samples from carcasses processed in the incriminated plant and isolated the outbreak strain. Additionally, carcass swabs were positive as well as pre-operational environmental swabs. The epidemiological findings led to a product recall.

An FSIS Update on Policy and Action to Prevent and Control Foodborne Disease Associated with Salmonella

Dr. Karen Becker DVM, MPH, DACVMPM

Applied Epidemiology Staff
Office of Public Health Science
Food Safety Inspection Service

The FSIS is the public Health agency in the USDA responsible for ensuring that nation's commercial supply of meat, poultry, and egg products is safe, wholesome, correctly labeled and packaged. FSIS develops microbiological performance standards designated by product class. In collaboration with public health partners, FSIS collects and evaluates epidemiological, microbiological, and traceback evidence during an outbreak investigation. Four objectives of an investigation include: implicating the food vehicle associated with illnesses, identifying the production establishment of origin, initiating control actions, and identifying root causes.

The largest outbreak attributed to a FSIS-regulated product was the Salmonella Heidelberg outbreak in chicken. Intensified sampling of the plant found high positive rates particularly in chicken parts. This called into question whether FSIS's verification sampling scheme could adequately monitor process control since the implicated establishments were considered Category 1.

Another outbreak investigation involved 2 clusters of Salmonella Enteritidis in Minnesota. The clusters were associated with consumption of frozen, raw, stuffed and breaded chicken products. Salmonella was found in in both establishments. These outbreaks further highlighted the problem with foods that are not cooked or partially cooked, but have the appearance of cooked.

In 2014 FSIS targeted Food Safety Assessments towards comminuted poultry establishments to increase understanding of interventions in use. FSIS conducted sampling to estimate prevalence of Salmonella in raw chicken parts and comminuted poultry. The resulting data was used to revise performance standards for these product categories. FSIS is drafting responses to comments requested in 80 FR 3940 and will consider changes on the proposed performance standards in chicken parts, comminuted chicken and turkey. These should be published in early 2016.

Annual Salmonella Report

Brenda Morningstar-Shaw

Diagnostic Bacteriology Laboratory
National Veterinary Services Laboratories

***Salmonella* serotypes isolated from animals in the United States: January 1-December 31, 2014**

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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. This report summarizes *Salmonella* serotyping submissions to NVSL from January 1 through December 31, 2014. *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, condor, crow, finch, hawk, goose, sparrow, partridge, parrot, parakeet, pheasant, pigeon quail, duck, and owl), Cattle, Chicken, Dog/Cat, Horse (horse, donkey, mule), Other Domestic (alpaca, ferret, goat, sheep, guinea pig, llama, mink), Pigs, Reptiles/Amphibians (iguana, lizard, reptile, snake, turtle, tortoise, amphibian, frog, alligator, crocodile), Turkey, Wild/Zoo (antelope, deer, fish, marine mammals, opossum, rabbit, raccoon, rodent, camel, monkey, lemur, tiger, zebra, rhinoceros, wallaby, cervid, cheetah, coyote, gazelle, jaguar, leopard, lion, warthog), and Other (environment, unknown).

Salmonella serotyping at the NVSL is an ISO 17025 accredited test Salmonellae are typed using polyvalent and single factor antisera to determine the O and H antigens. Approximately 60% of the sera used at the NVSL is produced in house as previously described. (Ewing) The remaining antisera are purchased from commercial vendors. All sera are subject to extensive quality control testing prior to use. *Salmonella* antigenic formulae are determined 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.

In 2014, 15,353 submissions were received for *Salmonella* serotyping. *Salmonella* isolates were divided into clinical isolates (4897), non-clinical isolates (6687), research and other (3769). Isolates that were submitted for *S*. Enteritidis or *S*. Heidelberg rule-out testing are included in the clinical and non-clinical counts. The sources of clinical and non-clinical *Salmonella* isolates are shown in Table 1. There were 289 different serotypes identified in 2014. Table 2 lists the 10 most common serotypes when all animal sources were combined. The most common isolates from chickens, turkeys, pigs, cattle, and horses are listed in Tables 3-7.

The NVSL provided a *Salmonella* Group D proficiency test to assess the ability of laboratories to isolate *Salmonella* from environmental samples and determine the serogroup (specifically group D) of any *Salmonella* isolated. The test consisted of 10 lyophilized cultures containing various combinations of *Salmonella* and common contaminants that simulated an environmental swab. The 2014 test included *Salmonella* serotypes Enteritidis, Javiana, Anatum, Oranienburg, Heidelberg, and an *sdf* negative Enteritidis. Contaminant bacteria included *Enterobacter cloacae, Citrobacter sedlakii*, *Citrobacter amalonaticus, Citrobacter freundii, Pseudomonas aeruginosa, and Providencia rettgeri*. Laboratories were instructed to test the samples according to the procedures used in their laboratories. The NVSL randomly retained 11% of the test kits and tested them blindly for QA purposes. The results of the proficiency test are shown in Table 8.

Additionally, the NVSL offered a *Salmonella* serotyping proficiency test to allow laboratories to assess their ability to serogroup or serotype *Salmonella*. The panel consisted of 10 pure *Salmonella* isolates, including *Salmonella* serotypes Berta, Saintpaul, Montevideo, Pensacola, Idikan, Essen, Liverpool, Fresno, Lille, and Enteritidis. Participants were given the option to perform serogrouping, partial serotyping, or full serotyping of the isolates and were graded based on appropriate identification to the level of typing they performed. The NVSL randomly retained 15% 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 2014

Source	**No. Clinical Submissions**	**No. Non-Clinical Submissions**
Cattle	1,603	290
Chicken	220	4,468
Horse	305	201
Swine	1,790	181

Turkey	305	883
All others	674	664
Total	**4,897**	**6,687**

Table 2: Most common serotypes in 2014: All sources

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Typhimurium	683	Senftenberg	1,478
4,(5),12:i:-	489	Mbandaka	545
Cerro	401	Kentucky	525
Dublin	342	Enteritidis	311
Agona	197	Cerro	273
Derby	196	Typhimurium	254
Montevideo	180	Montevideo	253
Senftenberg	160	Anatum	232
Newport	136	Braenderup	211
Infantis	135	Newport	135
All others	1,978	All others	2,470
Total	**4,897**	**Total**	**6,687**

Table 3: Most common serotypes in 2014: Chickens

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Enteritidis	86	Senftenberg	1106
Kentucky	30	Mbandaka	473
Infantis	13	Kentucky	450
Typhimurium	11	Enteritidis	291
Senftenberg	9	Typhimurium	93
All others	71	All others	2055
Total	**220**	**Total**	**4468**

Table 4: Most common serotypes in 2014: Turkeys

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Senftenberg	87	Senftenberg	271
Heidelberg	37	Anatum	96
Albany	29	Hadar	93
Ouakam	22	Muenster	74
Montevideo	16	Agona	52
All others	114	All others	247
Total	**305**	**Total**	**833**

Table 5: Most common serotypes in 2014: Pigs

Clinical		**Non-Clinical**	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
4,(5),12:i:-	383	Typhimurium	28
Typhimurium	332	4,(5),12:i:-	23
Derby	194	Derby	20
Agona	137	Bovismorbificans	18
Infantis	93	Havana	10
All others	651	All others	82
Total	**1790**	**Total**	**181**

Table 6: Most common serotypes in 2014: Cattle

Clinical		Non-Clinical	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Cerro	375	Cerro	95
Dublin	325	Montevideo	34
Typhimurium	174	Typhimurium	22
Montevideo	138	Newport	18
Newport	64	Dublin	17
All others	527	All others	104
Total	**1603**	**Total**	**290**

Table 7: Most common serotypes in 2014: Horses

Clinical		Non-Clinical	
Serotype	**No. Isolates**	**Serotype**	**No. Isolates**
Typhimurium	54	Typhimurium	56
Javiana	28	Newport	27
Newport	23	Anatum	19
Anatum	22	4,(5),12:i:-	10
Rubislaw/Thompson	12	Bovismorbificans	9
All others	154	All others	80
Total	**305**	**Total**	**201**

Table 8: Summary of NVSL *Salmonella* Group D proficiency test

	2010	**2011**	**2012**	**2013**	**2014**
Participants	55	70	73	61	80
Mean Score	92%	97%	92%	94%	98%
Score Range	100-44%	100-85%	100%-29%	100-68%	100-80%
Below Passing	3	0	N/A*	N/A**	0

Because of the change in grading method, a pass/fail designation was not assigned.
*2012 Seven individuals scored less than 80%
**2013 Four laboratories scored less that 80%

Table 9: Summary of NVSL *Salmonella* Serotyping proficiency test

	Serogrouping 2012	**Serotyping 2012**	**Serogrouping 2013**	**Serotyping 2013**	**Serogrouping 2013**	**Serotyping 2014**
Participants	22	13	18	14	34	23
Mean Score	98%	92%	98%	98.50%	99%	95%
Score Range	100-90%	100-70%	100-90%	100-90%	100-80%	100-80%

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.

The FDA Egg Safety Rule: Progress and Update

June deGraft Hanson DVM, PhD

Office of Food Safety
Division of Dairy, Egg and Meat Products
Egg and Meat Products Branch
Food and Drug Administration

The FDA's Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage, and Transportation Rule (The Egg Safety Rule) is an effort to reduce the incidence of SE in shell eggs. The Rule is applicable to producers with 3,000 or more laying hens who produce eggs for the table market and do not sell all eggs directly to consumers. The Rule requires producers to register with the FDA and to have a working SE Plan. It requires that producers acquire pullets that are National Poultry Improvement Plan (NPIP) SE-monitored, have methods in place to control for rodents and pests, have a biosecurity plan, have an effective Cleaning and Disinfection (C&D) Program, have a system to ensure cooling of eggs within 36 hours of lay, be able to test the environment for SE at specific ages of birds, and maintain records till at least a year after flock depopulation.

FDA has been conducting farm inspections since the rule became effective in 2010. The two types of inspections are Targeted inspections which consist of walkthrough of layer house and record review, and Comprehensive inspections, which include environmental sampling in addition to walkthrough and record review. Approximately 60% of registered farms have been inspected by the end of 2014. Between 2011-2014 the majority of the inspections were classified as No Action Indicated (NAI), or Voluntary Action Indicated (VAI). Only about 4% of inspections were classified as Official Action Indicated (OAI). During the same period, 735 environmental samples were collected from 235 firms. Twenty-four samples from 22 firms were positive for *Salmonella.*

Due to the outbreak of Highly Pathogenic Avian Influenza (HPAI) in the spring, FDA suspended egg farms inspections after discussions with federal and state agencies as well as other stakeholders; and a Biosecurity Directive to all field investigators with heightened biosecurity measures was issued. FDA is also revising its FD 107 Course accordingly for field investigators.

FDA received over 3000 comments on the drafted Guidance for producers of layers with outdoor access. The comments have all been reviewed and the Guidance is currently being revised.

FDA is also working on several outreach materials to be shared with industry. Lastly, FDA plans to conduct 100 targeted inspections, and 50 comprehensive inspections in FY2016.

How an open access USDA Intergenic Sequence Ribotype (ISR) database may facilitate routine serotyping of *Salmonella enterica* from farm-to-fork

Dr. Jean Guard DVM, PhD

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Serotyping of the food borne pathogen *Salmonella enterica* by the Kauffman-White-LeMinor (KWL) scheme has been the fundamental method applied for conducting epidemiological investigations since approximately 1950 (Edwards and Kauffmann 1952; Grimont and Weill 2007; Le Minor and others 1982). The Centers for Disease Control (CDC) has compiled data on serotypes associated with food borne disease over the last 42 years (CDC-NCZEID 2013). Despite its history as a useful typing scheme, the KWL scheme has major problems. Private laboratories now charge about $200 per sample, government supported laboratories charge at least $40 per sample, high quality antisera is difficult to produce and becoming less available, lot variation in antisera exist, failure to serotype is common because target O- and H-antigens might not be expressed, mixtures of *Salmonella* serotypes in a culture contribute to false identifications, and classification of reactions according to agglutination reactions varies between operators. In our experience, turn around time for larger groups of samples can exceed three months and yield less than 80% of samples as a named serotype.

Our laboratory receives field isolates of *Salmonella enterica* from around the world. Non-motile strains of what was submitted as serovar Pullorum were found to be serovar Enteritidis (Guard-Petter 1997). The number of submitted samples that were either misidentified or later classified as Pullorum and Gallinarum

were also of concern, because of the risk associated with shipping regulated serovars out to testing laboratories for definitive serotyping. Research objectives and safety issues thus made it imperative to develop an **in-house** method for distinguishing avian-adapted serovars such as Pullorum and Gallinarum from serovar Enteritidis, which is the world's leading cause of food borne salmonellosis in humans. DNA-based methods were being produced on a number of fronts, but either knowledgeable individuals from the CDC had not yet found them to be a replacement for KWL, they were not accessible and remained experimental, or they continued to be cost prohibitive often involving major equipment purchases.

Ribotyping has been a useful method for identification of the genus and species of bacteria, and thus further refinement was pursued to discriminate between closely related D1 serotypes of *Salmonella enterica* such as serovars Pullorum, Gallinarum and Enteritidis. An initial assessment of the 7 ribosomal regions of *Salmonella enterica* indicated that one of them, namely the rrnH region located near the gene dkgB close to the 299,000 base pair marker, had exceptional sequence heterogeneity (Morales and others 2006). The region was eventually defined as beginning at the first nucleotide after the 23S ribosomal gene and ending the base pair before the start of transfer RNA gene aspU (Figure 1)(Guard and others 2012). It included a 5S gene in the middle plus flanking regions. Size of the region varied from about 250 to 550 bp depending on serotype. Not only did the region yield sequence that could distinguish between serovars Pullorum, Gallinarum and Enteritidis, it appeared to produce sequence specific to nearly all serotypes. Results were also confirmed by conducting DNA microarray hybridization as well as submission for KWL when needed. The sequencing approach is called dkgB-linked intergenic sequence ribotyping (ISR). The current database contains 187 ISR sequences, which includes the top 30 serotypes linked to food borne illness by the CDC. A commercial source incorporates the assay (http://www.neogen.com/FoodSafety/NS_Sal.asp), but for many laboratories cost can be further contained by learning to do the assay in-house.

After development of the database, ISR was applied with collaborators sponsored by a South American cooperative of veterinarians and avian specialists (AMEVEA) in 3 different countries. ISR revealed the complexity and uniqueness of serotype composition in each study. We suggest that widespread and frequent application of ISR for routine monitoring of *Salmonella* on-farm by producers, occurring in addition to regulatory requirements, is possible. Developing knowledge of individualistic on-farm ecology might help identify emerging issues with the top 30 serotypes causing food borne illness, improve vaccination strategies, and alert producers to risk.

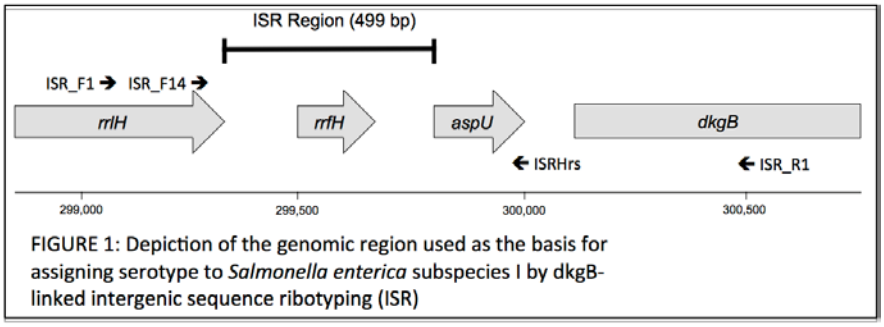

FIGURE 1: Depiction of the genomic region used as the basis for assigning serotype to *Salmonella enterica* subspecies I by dkgB-linked intergenic sequence ribotyping (ISR)

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Just When you Think you Have Salmonella Figured out …

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A poultry breeder company decided to qualify for the NPIP U.S. Salmonella Enteritidis Clean classification. This resulted in testing each of the companies flocks in a short period of time and then subsequently every 30 days. The Salmonella results for the last 5 years were analyzed to determine the relative rates for farms, flocks and even individual houses. Briefly, the Salmonella rate for the entire company has decreased dramatically. Even though the company has a Salmonella reduction plan, individual farms and even flocks on those farms have shown considerable variably. The remarkable findings were in the serotypes on the farms. It was not unusual for isolate 12 or more Salmonella serotypes from one flock (Figures 1 and 2). Many of these serotype introductions never established themselves in the house and were not found subsequently (Figure 3). Even more remarkable was the fact that different serotypes were isolated from each of the houses on the same farm.

Figure 1. Fifteen different serotypes isolated from a single flock on a farm. Three serotypes isolated from both houses, but 7 sere isolated only in house 1 and 5 others only in house 2.

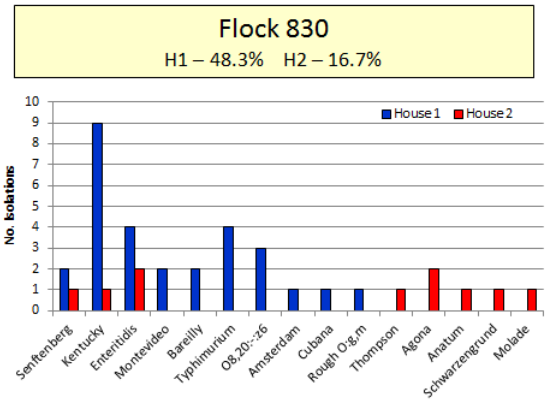

Figure 2. Fourteen different serotypes isolated from a single flock on a farm. Five serotypes isolated from both houses, but 3 were only isolated in house 1 and 6 only isolated in house 2.

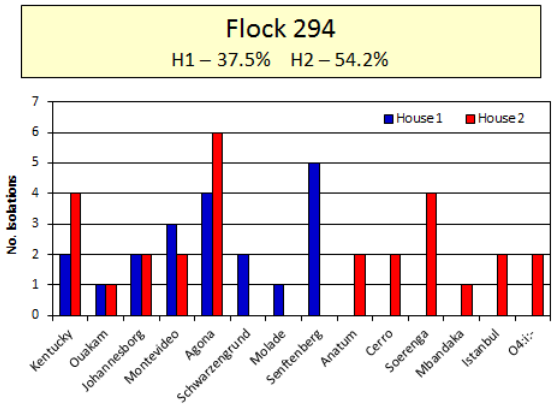

Figure 3. The results of the monthly Salmonella test on a farm showing the serotypes isolated. Notice some serotypes may be isolated multiple time, but most of them get introduced and then never detected again.

Flock 294

Age (wks)	House 1 (37.5%)	House 2 (54.2%)
24-27	Senftenberg	Montevideo •
28-31	Senftenberg Schwarzengrund	Agona • Anatum Johannesburg •
31	25 birds cultured: 1/75 cultures Salmonella positive Montevideo (1)	
32-35	Negative	Cerro
36-39	Ouakam Johannesburg •	Ouakam Soerenga
40-43		
44-47	Montevideo • Kentucky	Mbandaka Kentucky
48-51	Montevideo Molade	Istanbul
52-55	Agona •	Agona
56-59	Negative	O4:i:-

NPIP Report

In the past the annual report of the NPIP as it relates to Salmonella has been published by this committee, however it is redundant as it is also published in similar detail in the Transmissible Diseases of Poultry Committee. Therefore I refer to anyone interested to their report.

Committee Business:

The Committee did not discuss or put forth any Recommendations or Resolutions. The Chair announced that he and the Vice-Chair had completed their 5 year term and would be rolling off. Dr. Donna Kelly and Dr. Shelley Rankin both of the University of Pennsylvania volunteered to take the Chair and Vice-Chair roles, respectively. Their names have been submitted to the Executive Council for Approval.