

REPORT OF THE COMMITTEE ON SALMONELLA

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The Committee met on October 22, 2013 at the Town and Country Hotel, San Diego, California, from 8:00 a.m. – 12:00 p.m. There were 22 members and 22 guests present. After the Chair opened the meeting and welcomed the attendees, he reminded those present to sign the attendance sheets and if a member to check to see that their contact information was correct and if they were not members to 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.

Update: Outbreaks of Human Salmonella Infections Linked to Live Poultry from Mail-Order Hatcheries

Tara Creel Anderson DVM, MPH, PhD, CPH
EIS Officer, Outbreak Response and Prevention Branch, CDC

Outbreaks of *Salmonella* in live poultry linked to mail order hatcheries is a growing, but preventable public health issue. Backyard flocks are small scale poultry operations that typically consist of less than 50 birds. Poultry flocks may include chickens, ducks, turkeys, geese, or other avian species or combinations of all.

USDA sponsored an Urban Chicken Ownership study termed Poultry 2010, which surveyed four major cities. The study found that one percent of all households owned chickens. Four percent of households without chickens planned to have chickens within the next five years. Of the homeowners with chickens, the majority had been raising chickens less than five years. They cited the following reasons to own chickens: fun/hobby, better quality food, eggs and/or meat, animal welfare concerns, learning experience for kids, environmental concerns, and family traditions.

The mail-order hatchery industry in the United States consists of about 20 core hatcheries that sell more than 50,000,000 chicks annually. They distribute chicks nationwide typically through the U.S. Postal service and deliver directly to customers or to feed stores. The number of birds in a box varies by the size/type of bird. One box may contain multiple species, which provides a potential means of cross contamination. Because of the minimum purchase requirements for ordering chicks, most of these chicks are purchased at feed stores.

Eleven percent of human salmonellosis cases are due to animal contact or zoonotic sources. Recent (2006-2012) live poultry-associated outbreaks have included:

- Caterer contaminated food
- Chicken contact led to outbreak associated with infected delicatessen workers
- Poultry flock in daycare caused outbreak
- Death in nursing home resident, chicks had been brought into home
- Feed store employees became outbreak cases
- One sick postal worker who handled chicks in the mail

From 1990 to 2012 there have been 45 live poultry outbreaks involving 1,563 lab-confirmed cases, 221 hospitalizations and five deaths. The number of outbreaks and resulting cases has dramatically increased the last two years. For example, already this year there have been four outbreaks:

- Montevideo 12 cases 9 states
- Braenderup 50 21
- Multiple serotypes 145 28
- Typhimurium 328 37

The recent Typhimurium outbreak has involved the most cases and the largest area. PulseNet identified the outbreak in April 2013. Fourteen cases in nine states made up the initial report. The outbreak strain was a Typhimurium with a rare PFGE pattern. The characteristics of the persons infected showed the median age to be six, with 58% of the infected less than ten years old. Epidemiologic investigation included using foodborne disease questionnaires. The surveys pointed to exposure to live poultry. Ninety-seven percent of persons purchased poultry from feed stores.

The investigation highlighted some complex mail-order hatchery supply practices referred to as multiplying, trans-shipping, and drop shipping. These practices complicate the issue of tracing the sources of specific flocks involved in outbreaks.

The particular Typhimurium outbreak, found 97% of the poultry were purchased from 113 feed stores representing 33 companies. There were 18 sources of baby poultry. Mail-order hatchery A located in New Mexico was primarily the hatchery implicated in the outbreak.

Update on USDA, Agricultural Research Service (ARS) and Food and Drug Administration (FDA), National Antimicrobial Resistance Monitoring System (NARMS) Studies

Eileen Thacker, DVM, PhD, DACVM

National Program Leader, Animal Health and Acting Co-National Program Leader, Food Safety, USDA-ARS

The presentation covered the new changes – some already implemented and some proposed in NARMS sampling by the USDA and FDA. NARMS is a national public health surveillance system that tracks antibiotic resistance in foodborne bacteria. The NARMS program is a partnership between FDA, CDC and USDA and monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats and food animals.

New procedures implemented by FDA include monitoring animals in slaughter houses in a more random fashion than the current system which is heavily skewed towards problem farms. In addition, FDA is proposing the use of cecal (intestinal samples) as a measure of on-farm antibiotic resistance patterns. In addition to the use of cecal samples, an on-farm pilot project encompassing sampling feces from animals on the farm and following the same animals to slaughter. Species included in the study include beef and dairy cattle, swine and broilers and turkeys. This project is finishing the sample collection phase and it is anticipated there will be results in early 2014. In addition, a study by Dr. Paula Cray involving testing swine for *Salmonella* was briefly described.

Salmonella in Agricultural Exhibitions and Feed Stores in Colorado

Kristy Pabilonia, DVM, PhD, DACVM

Colorado State University

The Centers for Disease Control and Prevention reported eight *Salmonella* outbreaks linked to contact with live backyard poultry in 2012. With the number of backyard flocks increasing in the United States, evaluating the epidemiology of *Salmonella* in these flocks is important to understanding measures that can be utilized to prevent transmission of the bacteria between flocks and prevent zoonotic transmission to humans. Colorado State University and collaborators recently conducted two studies in an effort to further understand this issue.

The aim of the first study was to measure the frequency of isolation of *Salmonella* from the environment of poultry exhibits at agricultural fairs. The results are published in *Zoonoses and Public Health* (KL Pabilonia, KJ Cadmus, et al. Environmental *Salmonella* in agricultural fair poultry exhibits in Colorado. *Zoonoses and Public Health*, epub ahead of print 2013). Poultry cage litter; feed and environmental samples (floors and tables) were collected from 11 agricultural fairs. *Salmonella* was detected in 91% of fairs and 50.9% of all samples detected. Eleven *Salmonella* serotypes were detected, including Enteritidis, Infantis, Kentucky, and Braenderup (see table below). Results demonstrate that

environmental surfaces of agricultural fairs can be contaminated with *Salmonella* and could potentially serve as a route of transmission to bird owners and the general public.

Serotype	No. isolates		Serotype	No. isolates
Kentucky	13		Cubana	1
Meleagridis	4		Derby	1
Bredeney	3		Enteritidis	1
Infantis	2		Montevideo	1
O8,20:-:z6	2		Thompson	1
Braenderup	1			

The aim of the second study was to assess the prevalence of *Salmonella* in baby poultry enclosures at feed stores. Cage litter and drag swabs were collected from 30 feed stores. *Salmonella* was detected in 63% of the stores and 40% of the samples. All total 13 serotypes were identified (see table below). Feed stores sourced baby poultry from ten different hatcheries in seven states. Results of this study are currently being submitted for publication.

Serotype	No. Stores	No. Samples		Serotypes	No. Stores	No. Samples
Anatum	1	1		Montevideo	3	3
Anatum var 15+	3	7		Rough O:e,h:1,6	1	1
Braenderup	2	4		Senftenberg	3	14
Enteritidis	2	3		Typhimurium	6	24
Hadar	1	4		4,12:i:-	1	3
Infantis	2	3		3,19:NM	1	3
Kentucky	2	7		Montevideo	3	3

National Veterinary Services Laboratories (NVSL) Salmonella Update

Brenda Morningstar-Shaw

Diagnostic Bacteriology Laboratory, NVSL, USDA-APHIS-VS

The number of isolates submitted over the last five years as they are generally categorized is shown in the table below. The number of isolates submitted to the lab over the last 4 years has remained fairly consistent.

Year	No. isolates submitted	No. Clinical	No. Non-Clinical	Research	Misc.	No. Serotypes
2008	20,735	9328	7640	3117	650	298
2009	15,198	5278	8119	1321	480	399
2010	14,164	4700	8473	393	561	335
2011	15,977	6589	6810	1808	770	289
2012	14,750	4794	7742	1688	526	300
Total	80,824	30,689	38,784	8327	2987	

The top five serotypes from all sources is shown below. For clinical isolates there has been an increase (or moving up in rank) for 4,[5],12:i:-, Agona and Derby. For the Non-clinical isolates Heidelberg, Senftenberg, and Typhimurium have increased.

<p>Most Common Serotypes – All Sources 2012</p>
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Rank	Clinical	Non-Clinical
1	Typhimurium	Kentucky
2	4,[5],12:1:-	Enteritidis
3	Dublin	Heidelberg
4	Agona	Senftenberg
5	Derby	Typhimurium

With respect to the most common isolates in chickens, Muenchen has increased in the clinical isolate category and Heidelberg, Enteritidis, Senftenberg, and Mbandaka have all increased in the non-clinical area. For turkeys, Typhimurium and Saintpaul increased clinically, and Senftenberg, Muenster, Kentucky, and London increased in the non-clinical category while Hadar decreased.

Most Common Serotypes – Chickens 2012		
Rank	Clinical	Non-Clinical
1	Enteritidis	Heidelberg
2	Kentucky	Kentucky
3	Typhimurium	Enteritidis
4	Rough O:g,m:-	Senftenberg
5	Muenchen	Mbandaka

Most Common Serotypes – Turkeys 2012		
Rank	Clinical	Non-Clinical
1	Senftenberg	Senftenberg
2	Albany	Muenster
3	Typhimurium	Hadar
4	Saintpaul	Kentucky
5	Montevideo	London

With respect to the other species, isolates were not separated into clinical and non-clinical because there were very few non-clinical isolates. Therefore they were combined into one category. The ranking of isolates did not change with the cattle and swine isolates. For horses, Typhimurium, Anatum, and 4,[5],12:i:- increased while Newport decreased. With dogs and cats, Newport, Infantis, Agona, and 4,[5],12:i:- increased while Typhimurium decreased.

Rank	Cattle	Horses	Swine	Dogs/Cats
	All Sources	All Sources	All Sources	All Sources
1	Dublin	Typhimurium	Typhimurium	Newport
2	Typhimurium	Newport	Derby	Infantis
3	Cerro	Anatum	Agona	Typhimurium
4	Montevideo	Norwich	4,[5],12:i:-	Agona
5	Newport	4,[5],12:i:-	Infantis	4,[5],12:i:-

Frequently isolates that are known to be group D are submitted to determine if they are Enteritidis. Of 589 group D isolates, 566 (96%) were serotype Enteritidis. The remainders were Javiana, Berta, Ouakam, Alabama, and Miami. This is significant because there are some rapid tests that are only group D specific. Therefore they must be confirmed to be Enteritidis.

NVSL has implemented the xMAP Molecular Salmonella serotyping assay that was developed by CDC. The xMAP assay will not serotype all isolates so NVSL will maintain the ability to perform the gold standard of antisera-based conventional serotyping. Currently they are testing some isolates with the xMAP assay, with the goal to initially screen all isolates with the xMAP assay and then complete serotyping with antisera.

The molecular assay is faster and less cumbersome than conventional serotyping. In addition, it has high throughput, eliminates some sera QC issues and subjective interpretation. It also gives a genotype of the isolate, so the specific antigen can be detected even if it is not expressed. Lastly, it is less labor intensive. The negatives include the expense of the equipment and variable reagent cost.

The molecular typing assay is used for the SE rule out testing for the FDA Egg Rule testing. The assay provides a rapid turnaround, can type rough, weakly motile and even non-motile isolates. Additionally, the molecular assay detects the *sd*f gene which is specific to Enteritidis.

The NPIP Group D proficiency test and serotyping proficiency panel results have not been completed, but should be released in a few weeks.

Salmonella: What are others doing on-farm? What is the prevalence in the U.S.?

Annette O'Connor, BVSc, MVSc, DVSc, FACVSc(Epi)
Iowa State University

The presentation consisted of two parts: first to describe mandated Salmonella specific pre-harvest control approaches in major pork exporting countries and second to collate estimates of the prevalence of Salmonella pre-harvest in the United States.

A survey of Salmonella programs of various countries generally concludes that on-farm interventions have either not been effective in lowering Salmonella contamination or if it was somewhat effective it was shown to not be cost effective. The processing plant has been shown to be highly effective in controlling Salmonella contamination.

Salmonella Contamination in Beef Production

Dayna Harhay, PhD

Meat Safety and Quality Research Unit, USDA, ARS

A summary of 152 *Salmonella* outbreaks representing 12,181 illnesses from 1998-2011 showed that only 4% of the confirmed cases were attributed to contaminated beef. On the other hand, 28% were due to Tomatoes/lettuce, 12% nuts, and 9% sprouts, herbs and spices. One study looking at the prevalence of *Salmonella* in ground beef found a level of 4.2%. The most commonly identified serotypes were Montevideo, Anatum, Muenster, and Mbandaka. The peculiar aspect of those findings was although Montevideo is the most commonly isolated serotype in ground beef there has only been one outbreak due to Montevideo, whereas there have been six outbreaks of Typhimurium and Newport.

Apparently there are two sources of *Salmonella* contamination of beef, external and internal. External contamination is due to contaminated trim or some intervention failure. A study of over 3,000 carcasses found that at the beginning of processing the contamination level was 90%, after the hide was removed the level was 50%, but at the end of processing the level was less than 1% showing the effectiveness of the interventions in the plant.

Internal contamination may originate from fat trim containing lymph nodes that harbor *Salmonella*. Several studies have provided evidence to this route of contamination. A survey of the serotypes found in lymph nodes showed the top two serotypes were Montevideo (44%) and Anatum (25%), whereas Newport and Typhimurium were in only 1% and 0.4%, respectively. The serotypes in ground beef were Montevideo (21%) and Anatum (15%), whereas Typhimurium and Newport were 4.5% and 1.7%, respectively.

There is evidence that the internal contamination of the lymph nodes may originate from wound infections, whether from trauma or from biting insects. *Salmonella* infects the site of injury and then migrates to the regional lymph node.

Dr. Harhay described the variation in the virulence of *Salmonella* serotypes and strains to show that "not all *Salmonella* are created equal" or at least have equal ability to contaminate and cause infection. Her group is involved in a large project looking at the genetic basis for the virulence of *Salmonella* and the differences they are seeing between what is found in ground beef and in human cases. They are going to do whole genome sequence analysis on dozens of isolates.

National Poultry Improvement Plan (NPIP) Status Report

Dr. Denise Brinsen

Senior Coordinator NPIP, USDA-APHIS-VS

(Dr. Brinsen was unable to attend and sent this report in to be included in our report.)

Pullorum-Typhoid Status:

There were no isolations of *Salmonella pullorum* in commercial poultry in FY 2011 or FY 2012. There were two isolations of *Salmonella pullorum* in backyard birds in FY 2011. There were no isolations of *Salmonella pullorum* in any type of poultry in FY 2013. There have been no isolations of *Salmonella gallinarum* since 1987 in any type poultry in the U.S.

Hatchery Participation in the National Poultry Improvement Plan Testing Year FY2013	
Egg and Meat-Type Chickens: Participating	250
Turkeys: Participating	33
Waterfowl, Exhibition Poultry and Game Birds: Participating	788

Egg-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2013	
U.S. Pullorum-Typhoid Clean Flocks	262
Birds in Flocks	8,287,331
Birds Tested	42,889

Meat-Type Chicken Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2013	
U.S. Pullorum-Typhoid Clean Flocks	6,286
Birds in Flocks	100,100,705
Birds Tested	239,726

Turkey Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2013	
U.S. Pullorum-Typhoid Clean Flocks:	503
Birds in Flocks	4,754,650
Birds Tested	18,356
Waterfowl, Exhibition Poultry, and Game Birds Breeding Flocks in the National Poultry Improvement Plan Participation and Testing Summary Testing Year FY2013	
U. S. Pullorum-Typhoid Clean Flocks	6,001
Birds in Flocks	1,764,432
Birds Tested	147,980

U.S. <i>Salmonella enteritidis</i> Clean Egg-Type Breeding Chickens No. of flocks and birds in flocks by State with <i>Salmonella enteritidis</i> isolates, 1990-2013			
State	Environmental	Dead Germ	Birds
Arkansas			

Flocks	1		2
Birds in Flocks	6,000		15,000
Georgia			
Flocks	3	2	
Birds in Flocks	30,400	46000	
Illinois			
Flocks	3	2	1
Birds in Flocks	3,900	3700	1200
Indiana			
Flocks	15	2	1
Birds in Flocks	158,345	27,479	15,092
Kentucky			
Flocks	1		
Birds in Flocks	6,625		
Ohio			
Flocks	17		9
Birds in Flocks	192,700		91,600
Oregon			
Flocks	2		
Birds in Flocks	19,516		
Pennsylvania			
Flocks	16		6
Birds in Flocks	166,385		78,450
Texas			
Flocks	1		
Birds in Flocks	10,000		

Phage Type	Environmental	Dead Germ
Phage type 13		
Flocks	11	2
Birds in Flocks	152,000	3,700
Phage type 13A		
Flocks	5	2
Birds in Flocks	54,321	27,479
Phage type 2		
Flocks	2	
Birds in Flocks	28,900	
Phage type 23		
Flocks	21	
Birds in Flocks	16,000	
Phage type 28		
Flocks	2	2
Birds in Flocks	15,000	46,000
Phage type 34		
Flocks	2	
Birds in Flocks	12,500	
Phage type RND C		
Flocks	1	
Birds in Flocks	7,000	
Phage type-Untypable		
Flocks	2	
Birds in Flocks	24,000	
Phage type 8		

Flocks	21	
Birds in Flocks	237,701	

Egg-type Chicken breeding flocks with isolates of <i>Salmonella enteritidis</i> by phage type and by year 1989-2013		
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
2011	0	
2012	0	
2013	0	

Committee Business:

There was no old business, but a Resolution was brought before the Committee by Dr. Elizabeth Krushinskie. The title or subject matter of the Resolution was "Objection to Salmonella Linked to Human Illnesses Being Declared Adulterants. Following a brief discussion the Resolution was approved unanimously.