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Analysis of *Salmonella* serotypes from selected carcasses and raw ground products sampled prior to implementation of the Pathogen Reduction; Hazard Analysis and Critical Control Point Final Rule in the US

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Abstract

In July 1996, the US Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), published the Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems final rule to improve food safety of meat and poultry products. The final rule established, among other requirements, pathogen reduction performance standards for *Salmonella* for food animal carcasses and raw ground products. The final rule is to be fully implemented in three stages in successively smaller federally inspected meat and poultry slaughter and processing establishments. Implementation began in January 1998 and was completed in January 2000. Samples of carcasses of four species of food animals (cattle, swine, chickens, turkeys), and raw ground product from each of these species, were collected by FSIS from establishments prior to implementation of the final rule and cultured for *Salmonella*. This paper reports *Salmonella* serotype results of samples collected from June 1997 through August 1998. These results represent a baseline for future comparisons.

Keywords: *Salmonella*; HACCP; FSIS; Intervention

1. Introduction

In July 1996, the US Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), published the Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems final rule to improve food safety of meat and poultry

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products. The final rule established, among other requirements, pathogen reduction performance standards for *Salmonella* for food animal carcasses and raw ground products. Each slaughter establishment and each establishment producing raw ground products must ensure its *Salmonella* contamination rate is below the current national baseline prevalence.

Large establishments (establishments with 500 or more employees) were subject to this regulatory sampling in January 1998. Smaller establishments (with 10 or more employees, but fewer than 500) were implemented in January 1999. Very small establishments (fewer than 10 employees or annual sales of less than $2.5 million) will be implemented in January 2000.

This paper reports on the *Salmonella* serotype results of samples collected prior to implementation of the final rule in establishments of all sizes from June 1997 through August 1998.

2. Methods

Cattle and swine carcasses were randomly sampled at the end of the slaughter process in the cooler. Cattle carcasses were sampled by swabbing three 100-cm^2 sites (flank, rump, and brisket) with a sterile sponge moistened in 10 ml of chilled buffered peptone water (BPW). Swine carcasses were sampled by swabbing three 100-cm^2 sites (ham, belly, and jowls) with a sterile sponge moistened in BPW. After swabbing the three sites, the sponge was placed back into its bag and shipped at 0–10°C to FSIS laboratories for analysis.

Poultry carcasses were randomly sampled after the chill tank at the end of the drip line or the last readily accessible point prior to packing or cut up. Carcass sampling for poultry carcasses was a whole bird rinse. Chicken carcasses were rinsed in a sterile 3500-ml stomacher-type plastic bag with 400 ml of chilled BPW. At least 30 ml of the rinse fluid were decanted into a sterile sample container and shipped at 0–10°C to FSIS laboratories for analysis. The procedure for turkeys was similar with the exception that 600 ml of chilled BPW were used to rinse the birds.

Raw ground product samples were randomly selected and collected after the grinding process and, if possible, before any addition of spices or seasoning, but prior to final packaging. About one-half pound of raw ground product was aseptically collected in a plastic bag, chilled and transported at 0–10°C to the laboratory for culture.

Samples were shipped the same day they were collected to one of three FSIS laboratories in Athens, GA, St. Louis, MO, or Alameda, CA, for analysis. The samples were required to arrive at the laboratories cooled (0–10°C) but not frozen.

Samples were prepared for *Salmonella* analysis by the addition of: (1) 50 ml of BPW to the premoistened sponge sample; (2) 30 ml of BPW to 30 ml of poultry carcass rinse fluid; or (3) 225 of BPW to 25 g of raw ground product. The prepared samples were then analyzed according to culture procedures described in the FSIS Microbiology Guidebook (MLG), 3rd edition (USDA, FSIS, 1998). All presumptive positive samples were confirmed culturally, as described in the MLG. *Salmonella* isolates were sent to the USDA, APHIS National Veterinary Services Laboratories, Ames, IA, for serotyping.

Salmonella isolates were serotyped using procedures described in Edwards and Ewing's Identification of Enterobacteriaceae, 4th edition (Ewing, 1986). The cell wall antigen (somatic or 'O') was first identified using a saline suspension of cells mixed with antisera in a slide agglutination test. The flagellar ('H') antigen was then determined using a formalin-killed broth culture mixed with antisera in a tube agglutination test. Absorbed single factor sera were used to confirm the specific antigens in both 'O' and 'H' tests. After the first flagellar antigen was identified, a small tissue culture dish containing antisera mixed with motility media was inoculated with the isolate to force the *Salmonella* to change phase. The second phase was then identified in a tube agglutination test. Biochemical tests were done to identify any 'bioserotypes', such as *S. choleraesuis*. After all antigens were identified, the isolate was reported by the serotype name.

3. Results and discussion

3.1. Analysis of samples

The database contains eight different types of samples representing eight different types of products: cattle carcasses, swine carcasses, chicken car-

Table 1
Most common *Salmonella* serotypes found in sampled cattle carcasses and raw ground beef

Rank	Cattle carcasses			Raw ground beef		
	Serotype	*n*	(%)	Serotype	*n*	(%)
1	*montevideo*	38	10.4	*montevideo*	31	17.9
2	*typhimurium*	30	8.2	*anatum*	28	16.2
3	*muenster*	28	7.7	*typhimurium* (var. *copenhagen*)	20	11.6
4	*anatum*	26	7.1	*reading*	13	7.5
5	*typhimurium* (var. *copenhagen*)	23	6.3	*muenster*	8	4.6
6	*mbandaka*	22	6.0	*meleagridis*	7	4.0
7	*meleagridis*	22	6.0	*kentucky*	6	3.5
8	*kentucky*	16	4.4	*hadar*	5	2.9
9	*new-brunswick*	15	4.1	*typhimurium*	5	2.9
10	*derby, heidelberg*	12	3.3	*derby, give, infantis, newport, senftenberg*	4	2.3
	Total isolates serotyped	364		Total isolates serotyped	173	

casses, and turkey carcasses, and raw ground beef, raw ground pork, raw ground chicken, and raw ground turkey. Tables 1–4 show the results of serotyping 3717 isolates representing approximately half the number of *Salmonella* positive findings. These tables list the top 10 serotypes for each of the eight product types. Each table compares carcasses with raw ground product to better illustrate similarities in serotypes among the products.

The most common serotypes in cattle carcasses (Table 1) were *montevideo*, *typhimurium*, *muenster*, and *anatum*, while from raw ground beef the most common serotypes were *montevideo*, *anatum*, *typhimurium* (var. Copenhagen), and *reading*.

The most common serotypes from swine carcasses (Table 2) were *derby*, *johannesburg*, *anatum*, and *typhimurium* (var. Copenhagen). The most common serotypes in raw ground pork were *derby*, *typhimurium* (var. Copenhagen), *anatum*, and *heidelberg*.

The most common serotypes recovered from chicken carcasses (Table 3) were *heidelberg*, *kentucky*, *hadar*, and *typhimurium*. The most common serotypes in raw ground chicken were *heidelberg*, *kentucky*, *schwarzengrund*, and *infantis*.

The most common serotypes recovered from turkey carcasses (Table 4) were *hadar*, *heidelberg*, *agona*, and *senftenberg*. The most common serotypes

Table 2
Most common *Salmonella* serotypes found in sampled swine carcasses and raw ground pork

Rank	Swine carcasses			Raw ground pork		
	Serotype	*n*	(%)	Serotype	*n*	(%)
1	*derby*	244	27.7	*derby*	106	16.9
2	*johannesburg*	88	10.0	*typhimurium*	94	15.0
3	*anatum*	70	8.0	(var. *copenhagen*)		
4	*typhimurium*	62	7.0	*amatum*	45	7.2
	(var. *copenhagen*)			*heidelberg*	40	6.4
5	*infantis*	41	4.7	*infantis*	32	5.1
6	*saint-paul*	40	4.5	*agona*	29	4.6
7	*reading*	36	4.1	*reading*	25	4.0
8	*london*	30	3.4	*muenchen*	21	3.3
9	*heidelberg*	27	3.1	*typhimurium*	21	3.3
10	*typhimurium*	24	2.7	*london*	19	3.0
	Total isolates serotyped	880		Total isolates serotyped	628	

Table 3
Most common *Salmonella* serotypes found in sampled chicken carcasses and raw ground chicken

Rank	Chicken carcasses			Raw ground chicken		
	Serotype	*n*	(%)	Serotype	*n*	(%)
1	*heidelberg*	210	26.2	*heidelberg*	24	30.0
2	*kentucky*	157	19.6	*kentucky*	11	13.8
3	*hadar*	63	7.8	*schwarzengrund*	9	11.3
4	*typhimurium*	42	5.2	*infantis*	5	6.3
5	*typhimurium* (var. *copenhagen*)	39	4.9	*hadar*	4	5.0
6	*thompson*	38	4.7	*mbandaka*	3	3.8
7	*montevideo*	30	3.7	*thompson*	3	3.8
8	*schwarzengrund*	28	3.5	*agona*	2	2.5
9	*mbandaka*	16	2.0	*braenderup*	2	2.5
10	*senftenberg*	16	2.0	nine serotypes had	1	1.3
	Total isolates serotyped	803		Total isolates serotyped	80	

Table 4
Most common *Salmonella* serotypes found in sampled turkey carcasses and raw ground turkey

Rank	Turkey carcasses			Raw ground turkey		
	Serotype	Count	(%)	Serotype	Count	(%)
1	*hadar*	72	15.3	*hadar*	76	23.8
2	*heidelberg*	67	14.3	*agona*	28	8.8
3	*agona*	43	9.1	*muenster*	23	7.2
4	*senftenberg*	42	8.9	*senftenberg*	23	7.2
5	*muenster*	34	7.2	*heidelberg*	20	6.3
6	*arizona*	25	5.3	*typhimurium*	16	5.0
7	*schwarzengrund*	18	3.8	*reading*	15	4.7
8	*montevideo*	16	3.4	*schwarzengrund*	15	4.7
9	*saint-paul*	15	3.2	*saint-paul*	14	4.4
10	*bredeney, reading*	14	3.0	*brandenburg*	10	3.1
	Total isolates serotyped	470		Total isolates serotyped	319	

in raw ground turkey were *hadar*, *agona*, *muenster*, and *senftenberg*.

The most common serotypes on cattle carcasses (*montevideo*), swine carcasses (*derby*), chicken carcasses (*heidelberg*), and turkey carcasses (*hadar*) were also the most common serotypes in raw ground beef, raw ground pork, raw ground chicken, and raw ground turkey, respectively. Since raw ground product generally consists of meat from multiple animals such a finding is intuitively appealing.

The Centers for Disease Control and Prevention publishes an Annual Summary Tabulation which contains surveillance data on reported laboratory-confirmed *Salmonella* isolates in the US. The National *Salmonella* Surveillance System collects reports of isolates of *Salmonella* from human sources from every state in the US. This information is reported through the Public Health Laboratory Information System (PHLIS), an electronic reporting system, by the State Public Health Laboratory Directors and State and Territorial Epidemiologists to the Foodborne and Diarrheal Diseases Branch and the Biostatistics and Information Branch of the Division of Bacterial and Mycotic Diseases in the National Center for Infectious Diseases (Helfrick et al., 1997).

Under this system the most frequent human *Salmonella* serotypes reported for 1997 were *S. typhimurium* and *S. enteritidis*. These two serotypes accounted for approximately 47% of reported human cases. *S. typhimurium* was the second most common isolate from cattle carcasses and the fourth most common isolate from chicken carcasses. *S.*

typhimurium was also among the top 10 serotypes in all products, except turkey carcasses and raw ground chicken. *S. enteritidis* was not among the 10 most common isolates from any of the products.

Undoubtedly, foods of animal origin are a significant cause of *Salmonella* infections in humans (Tauxe, 1991; Morris, 1996) and Salmonellae found in animals are often isolated from humans (Ekperigin and Nagaraja, 1998, Troutt and Osburn, 1997). Nevertheless, the most common serotypes in the tested products were not the most common serotypes isolated from cases of human illness. The most common isolate from chicken carcasses and raw ground chicken was *S. heidelberg*. This was the third most common human isolate and accounted for 6.1% of all human isolates. *S. montevideo*, the most common isolate from raw ground beef and cattle carcasses was the seventh most common human isolate accounting for 2.1% of reported *Salmonella* isolates. *S. hadar* was the most common isolate from turkey carcasses and raw ground turkey. It accounted for 1.9% of human *Salmonella* isolates. *S. derby* was the most common isolate from swine carcasses and raw ground pork. It was the 27th most common human isolate and accounted for 0.4% of the isolates.

A plausible explanation for this discordance may be that some serotypes that occur less often in animals (*S. typhimurium*, *S. enteritidis*) could be more pathogenic for humans than serotypes that occur more often in livestock and poultry (e.g., *S. montevideo* in beef). Alternatively, the exposure doses of the serotypes that cause more human illnesses may be greater than the exposure doses of those serotypes that cause less human illnesses. Exposure dose is a function of the initial inoculum size and growth of bacteria post-production. Because the HACCP database does not provide bacterial enumeration, the initial inoculum in product is not known. Also, consumption patterns and handling and cooking practices vary for each of the sampled products. These patterns and practices were not examined in this analysis but could account for some of the discordance (Altekruse et al., 1998).

Of course, it is possible that there are sources for human *Salmonella* other than these products that might account for these discrepancies. For example, eggs are a source of *S. enteritidis* that is not captured in the HACCP pre-implementation data (Angulo, 1998; CDC, 1996).

4. Conclusions

In conclusion, the most common *Salmonella* serotypes found on animal carcasses were also the most common serotypes found in the corresponding raw ground product. The most common *Salmonella* serotypes found on meat and poultry products prior to the implementation of the final rule did not correlate well with those found most often to cause human illness.

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