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Research Paper

Confounding Role of *Salmonella* Serotype Dublin Testing Results of Boneless and Ground Beef Purchased for the National School Lunch Program, October 2013 to July 2017

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ABSTRACT

The Agricultural Marketing Service procures boneless and ground beef for federal nutrition assistance programs. It tests procured beef for concentrations of standard plate counts (SPCs), coliforms, and *Escherichia coli* and for the presence of *Salmonella* and Shiga toxin–producing *E. coli*. Any lot exceeding predefined critical limits (100,000 CFU g ^{1} for SPCs, 1,000 CFU g ^{1} for coliforms, and 500 CFU g ^{1} for *E. coli*) or positive for *Salmonella* or Shiga toxin–producing *E. coli* is rejected for purchase. Between 1 October 2013 and 31 July 2017, 166,796 boneless beef lots (each approximately 900 kg) and 25,051 ground beef sublots (each approximately 4,500 kg) were produced. *Salmonella* was detected in 1,955 (1.17%) boneless beef lots and 219 (0.87%) ground beef sublots. *Salmonella* sample size increased from an individual 25-g sample to a co-enriched 325-g sample on 1 March 2015. *Salmonella* presence was associated with season (lowest in spring), larger sample size, and increased log SPC in boneless and ground beef. Increased log *E. coli* was associated with *Salmonella* presence in boneless beef, but not ground beef. *Salmonella* Dublin was the most common serotype in boneless beef (743 of 1,407, 52.8%) and ground beef (35 of 171, 20.5%). *Salmonella* Dublin was generally associated with lower indicator microorganism concentrations compared with other *Salmonella* serotypes as a group. Relative to other *Salmonella, Salmonella* Dublin was associated with season (more common in spring) and smaller sample size in boneless and ground beef. Decreased log SPCs and log coliforms were associated with *Salmonella* Dublin presence in boneless beef, but not in ground beef. Differential associations between *Salmonella* Dublin and other serotypes with indicator microorganisms were strong enough to cause confounding and suggest that the presence of *Salmonella* Dublin needs to be accounted for when evaluating indicator performance to assess *Salmonella* risk in boneless and ground beef.

HIGHLIGHTS

Salmonella was associated with indicator microorganism detection and concentration.
Salmonella Dublin represented 52.8% of *Salmonella* in boneless and 20.5% in ground beef.
Salmonella Dublin was associated with lower indicator levels compared with other *Salmonella*.

Key words: Beef; National School Lunch Program; *Salmonella* serotype Dublin

Salmonella causes an estimated 1,000,000 cases, 19,000 hospitalizations, and 350 deaths annually in the United States *(33)*. *Salmonella* infection has a high economic burden, costing an estimated $3.7 billion annually in the United States *(15)*. There are more than 2,600 *Salmonella* serotypes and about 360 serotypes that cause human illness. Of these, 20 serotypes account for 69% of all human *Salmonella* illnesses *(4, 30)*.

Approximately 7% of human *Salmonella* infections are attributed to contaminated beef consumption *(18)*. Cattle may become infected in farm or lairage environments before or during transit to abattoirs *(22, 32)*. Beef intended for human consumption can become contaminated during the production process if it comes into contact with *Salmonella* on cattle hides, colonized lymph nodes, or feces or through cross-contamination of production surfaces and tools *(2, 20, 40)*. In recent years, producers and regulators instituted large-scale ground beef recalls because of foodborne outbreaks associated with *Salmonella* contamination *(11)*. In 2018, for example, a large outbreak of *Salmonella* Newport involving 403 illnesses and 117 hospitalizations across 30 states was linked to ground beef consumption and resulted in a recall of 12 million lb of ground beef *(5)*.

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Salmonella Dublin is a cattle-adapted serotype and is associated with dairy cattle *(16)*. *Salmonella* Dublin was the most common bovine clinical isolate submitted to the National Veterinary Service Laboratory in both 2016 and 2017 *(25, 26)*. It was the second most common in 2015 *(21)*. *Salmonella* Dublin was the most prevalent *Salmonella* serotype in a large study of U.S. dairy cattle, mostly from Wisconsin, with clinical symptoms, accounting for 23% of isolates *(38)*. *Salmonella* Dublin is also commonly detected in nonclinical bovine samples. The National Veterinary Service Laboratory reported *Salmonella* Dublin was the fourth most common nonclinical *Salmonella* serotype in 2015 and 2017 *(21)*. It may also be found in lymph nodes at harvest *(40)*. An additional study of bulk milk tanks in New York found *Salmonella* Dublin in 4% of all samples and in 60% of all *Salmonella*-positive samples *(6)*.

Salmonella transmission between cattle typically occurs via the fecal-oral route but also rarely occurs via respiratory or conjunctiva routes *(13, 14, 27)*. *Salmonella* infections in pregnant cows can cross the placenta, leading to calf abortions *(13, 27)*. Clinical signs of *Salmonella* infections in cows include diarrhea, pneumonia, abortion, and death *(14, 38)*. Clinical severity varies with age and is most severe in cattle younger than 3 months, with neonatal diarrhea being a particular challenge *(27, 37)*. *Salmonella* Dublin carrier status is a well-documented problem in cattle *(6, 14)*. Carrier animals may maintain *Salmonella* Dublin in their lymph nodes or internal organs *(3, 13, 14)*. These animals may continue to shed *Salmonella* Dublin constantly or intermittently, or they may become latent carriers. The presence of carrier cattle with subclinical *Salmonella* Dublin infections complicates its controls on farms and ranches *(13, 14, 16, 22)*.

Salmonella Dublin has not been frequently associated with human illness and was not listed among the top 20 culture-confirmed human serotypes reported to the Centers for Disease Control and Prevention (CDC) in 2016 *(4)*. However, *Salmonella* Dublin infection incidence rates in humans have increased faster than rates of other *Salmonella* serotypes in recent years. A study determined *Salmonella* Dublin infections in humans were significantly more severe than *Salmonella* infections caused by other serotypes: infection with *Salmonella* Dublin was more likely to result in sepsis, more likely to require hospitalization, and more likely to cause death *(12)*. Furthermore, *Salmonella* Dublin incidence was higher than that of other *Salmonella* serotypes in adults older than 18 years, but a lower proportion of children aged 5 to 17 years were affected by *Salmonella* Dublin compared with other *Salmonella* serotypes in the United States.

Salmonella Dublin frequently displays antimicrobial resistance (AMR), with up to 79% of isolates being AMR *(12, 16, 38)*. AMR complicates treatment and leads to more serious illnesses. Furthermore, *Salmonella* Dublin AMR increased significantly over the last 20 years. Given the seriousness of *Salmonella* Dublin infection, its increasing incidence, and AMR prevalence, it is a notable human public health concern.

An outbreak of *Salmonella* Dublin in August 2019 resulted in 10 reported cases in the United States *(8)*. Of the 10 cases, 8 were hospitalized, and 1 died. The Centers for Disease Control and Prevention estimates that typical (non–Dublin-specific) *Salmonella* infections only result in hospitalization in approximately 20% of all cases. Further evidence that this outbreak led to more severe health outcomes than general *Salmonella* outbreaks was the *Salmonella* Dublin presence in blood samples for five cases. Cases presented in six states and occurred predominately among older men. These severe human health outcomes call for a more in-depth analysis of the *Salmonella* Dublin ecology.

Indicator microorganisms are used during beef and other meat production and processing to monitor food quality, hygiene, and process control *(31)*. Monitoring and controlling processes with these indicator organisms is intended to reduce the likelihood of pathogen presence *(1)*. However, multiple studies have shown a sporadic to weak correlation between presence of indicator microorganisms and *Salmonella (7, 9, 19)*. One study of the association between indicator microorganisms and *Salmonella* serotypes in lymph nodes found highly variable indicator concentrations by lymph node location and serotype, including *Salmonella* Dublin *(3)*.

Data and analysis of the U.S. Department of Agriculture Agricultural Marketing Service (AMS) boneless and ground beef purchasing programs from 2011 to 2018 determined *Salmonella* Dublin accounted for 51 and 17.2% of *Salmonella* isolates recovered from boneless and ground beef, respectively *(7, 39)*. These studies examined associations between contract-established critical limit exceedance and pathogen presence. Weak associations between critical limit exceedance and *Salmonella* presence were found. Potential associations between *Salmonella* presence and indicator microorganism concentrations on broader, continuous scales and potential variation associated with specific *Salmonella* serotypes have not been explored.

Here we examine AMS data for October 2013 through July 2017. Our goals are twofold. First, we describe associations between indicator microorganism concentration and pathogen presence, along with season and AMS purchase requirements. Second, we investigate *Salmonella* Dublin's unique relationship with these same factors relative to *Salmonella*-negative lots and to lots positive for other *Salmonella* serotypes.

MATERIALS AND METHODS

Sampling and analysis. Samples of boneless and ground beef scheduled for raw, uncooked delivery at school food service and similar facilities were collected and processed as previously described *(7, 39)*. Trained vendor personnel at each establishment collected samples using a scalpel excision or drill-sampling device for boneless beef and by grab sampling for boneless beef. Samples were packaged, placed on dry ice, and shipped overnight to AMS-designated laboratories for analysis in accordance with AMS purchase specifications *(34)*. AMS-designated laboratories performed all microbiological analyses according to the Food Safety and Inspection Service (FSIS) *Microbiological Laboratory Guidebook (36)*. Two FSIS and AMS testing protocol changes occurred in early 2015. First, the boneless beef *Salmonella* sample size was increased from 25 to 325 g *(35)*. Second, the *Salmonella* analysis method was changed from the individual 25-g sample to a 325-g co-enriched sample also used for *E. coli* O157:H7 testing *(35)*. AMS required its vendors to switch sampling methods in March 2015. *Salmonella* isolates recovered by AMS-designated laboratories were sent to the FSIS Eastern Laboratory for serotyping *(24)*. AMS maintains all results electronically.

More boneless beef than ground beef lots were sampled between 2013 and 2017, because AMS purchase specifications require testing of every 2,000 lb of boneless beef compared with every 10,000 lb of ground beef. In addition, boneless beef sublots found positive are discarded and new replacement sublots are tested before grinding. Lastly, some boneless beef is used without grinding.

Data analysis. Indicator microorganism and *Salmonella* data for 166,796 AMS boneless beef lots produced by 15 vendors and 25,051 resultant ground beef sublots produced by 10 vendors between 1 October 2013 and 31 July 2017 were analyzed. Critical limits were defined as 100,000 CFU g^{-1} for standard plate counts (SPCs), 1,000 CFU g^{-1} for coliforms, and 500 CFU g^{-1} for *E. coli*. All data analyses were conducted using STATA 14.2 *(34)*. Initially, univariable associations between indicator microorganism detection, season, and pathogen sample size with *Salmonella* presence were determined. Seasons were defined as spring (March to May), summer (June to August), fall (September to November), and winter (December to February). Spring was used as the seasonal referent category. Seasonal associations were determined using Pearson chi-square tests. The *Salmonella* sample size was defined as 25 g for lots produced before 1 March 2015 and as 325 g for lots produced on or after 1 March 2015. Boneless beef lots and ground beef sublots without detectable SPCs, total coliforms, or *E. coli* were considered to have concentrations of 0 log CFU g^{-1}.

The boneless beef and ground beef analyses considered three outcomes. The primary outcome of interest was *Salmonella* presence. *Salmonella*-positive samples were further stratified into two outcome groups. One outcome group consisted of all *Salmonella* Dublin isolates. The other outcome group consisted of *Salmonella* serotypes other than *Salmonella* Dublin. Associations between indicator microorganism detection (SPCs, total coliforms, and *E. coli*) and outcome (all *Salmonella* serotypes, *Salmonella* Dublin, or a serotype other than *Salmonella* Dublin) were determined using 2 by 2 tables to calculate odds ratios (ORs) and 95% confidence intervals (95% CI). Univariable associations among log SPCs, log total coliforms, and log *E. coli* with each outcome were determined using logistic regression to determine ORs and 95% CI. Multivariable associations between seasons, pathogen sample sizes, log SPCs, log total coliforms, and log *E. coli* with each outcome were determined using mixed-effects general linear modeling with nesting by the vendor. General linear modeling was completed using stepwise deletion of nonsignificant variables. Final models reported ORs and 95% CI and included only those variables significantly associated with outcome.

RESULTS AND DISCUSSION

***Salmonella* presence in boneless and ground beef.** *Salmonella* was present in 1,955 (1.17%) boneless beef lots (Table 1). *Salmonella* prevalence differed significantly in univariable analysis by season ($\chi^2_3 = 125.33$, $P < 0.001$) pathogen sample size (OR = 2.16, 95% CI: 1.96 to 2.39), and vendor ($\chi^2_{14} = 576.8$, $P < 0.001$). *Salmonella* prevalence was highest in the fall, followed by winter, summer, and spring. Of the 15 vendors, 13 produced at least one *Salmonella*-positive lot. *Salmonella* prevalence ranged by vendor from 3.41 to 0.00%. *Salmonella* was significantly more likely to be recovered using the larger sample size, with 1.54% of lots tested from March 2015 onward found positive for *Salmonella* compared with 0.72% lots produced before March 2015.

TABLE 1. *AMS vendor* Salmonella *performance*

	Boneless beef			Ground beef		
	No. of lots	*Salmonella* positives *n*	*Salmonella* positives %	No. of sublots	*Salmonella* positives *n*	*Salmonella* positives %
Overall	166,796	1,955	1.17	25,051	219	0.87
Season[a]						
Spring	23,647	156	0.66	3,500	18	0.51
Summer	31,873	325	1.02	4,514	59	1.31
Fall	58,085	887	1.53	8,831	73	0.83
Winter	53,191	587	1.10	8,206	69	0.84
Sample size[b]						
25 g	74,350	533	0.72	14,055	95	0.68
325 g	92,446	1,422	1.54	10,996	124	1.13

[a] Boneless beef: $\chi^2_3 = 125.3$, $P < 0.001$. Ground beef: $\chi^2_3 = 15.3$, $P = 0.002$.
[b] 325-g co-enriched sampling began 1 March 2015. Boneless beef: OR = 2.16, 95% CI: 1.96 to 2.39. Ground beef: OR = 1.68, 95% CI: 1.27 to 2.22.

During the same period, AMS vendors produced 25,051 sublots of ground beef scheduled for raw, uncooked delivery to school food service and similar facilities (Table 1). These sublots were produced by 10 vendors using AMS boneless beef that had met AMS indicator microorganism and pathogen testing purchase specifications. *Salmonella* was detected in 219 (0.87%) ground beef sublots. Prevalence differed significantly by season ($\chi^2_3 = 15.3$, $P = 0.002$), pathogen sample size (OR = 1.68, 95% CI: 1.27 to 2.22), and vendor ($\chi^2_9 = 198.39$, $P < 0.001$). *Salmonella* was most commonly found in ground beef during the summer, followed by winter, fall, and spring. All 10 vendors produced at least one *Salmonella*-positive ground beef sublot, and prevalence ranged from 3.37 to 0.06%. *Salmonella* was significantly more likely to be recovered from sublots produced after 1 March 2015 (1.13%) compared with those produced earlier (0.68%).

Average indicator microorganism concentrations were low but positively skewed in both boneless and ground beef. These concentrations remained skewed following log transformation (Table 2). Skewness was partly because of relatively high proportions of boneless beef lots and ground beef sublots containing nondetectable concentrations of indicator microorganisms. In boneless beef, 16.7% of lots had nondetectable SPCs, 91.7% had nondetectable total coliforms, and 98.1% had nondetectable *E. coli*. In ground beef, 21.1% of sublots had nondetectable SPCs, 87.2% had nondetectable coliforms, and 95.3% had nondetectable *E. coli*.

Indicator microorganism detection was consistently associated with *Salmonella* presence (Table 3). In boneless beef, *Salmonella* was significantly more likely to be

TABLE 2. *AMS vendor indicator organism performance*

	Concn (log CFU g^{-1})					
	Boneless beef			Ground beef		
Indicator[a]	SPCs	Total coliforms	*E. coli*	SPCs	Total coliforms	*E. coli*
Maximum	6.34	5.30	4.41	5.60	4.30	3.64
Q3	2.32	0.00	0.00	2.52	0.00	0.00
Median	1.78	0.00	0.00	1.95	0.00	0.00
Q1	1.00	0.00	0.00	1.00	0.00	0.00
Minimum	0.00	0.00	0.00	0.00	0.00	0.00

[a] Q3, third quarter; Q1, first quarter.

detected when SPCs (OR = 1.50, 95% CI: 1.30 to 1.72), total coliforms (OR = 1.34, 95% CI: 1.15 to 1.55), or *E. coli* (OR = 2.24, 95% CI: 1.76 to 2.81) were detected. In ground beef, *Salmonella* was significantly more likely to be detected when SPCs (OR = 1.62, 95% CI: 1.10 to 2.46), coliforms (OR = 2.79, 95% CI: 2.04 to 3.77), or *E. coli* (OR = 3.12, 95% CI: 2.02 to 4.65) were detected.

Increased indicator microorganism log concentrations were consistently associated with *Salmonella* presence in univariable analysis. In boneless beef, *Salmonella* detection was significantly associated with increased log SPCs (OR = 1.15, 95% CI: 1.10 to 1.20), log total coliforms (OR = 1.26, 95% CI: 1.14 to 1.40), and log *E. coli* (OR = 1.77, 95% CI: 1.53 to 2.06). In ground beef, *Salmonella* was also significantly associated with increased log SPCs (OR = 1.45, 95% CI: 1.27 to 1.67), log total coliforms (OR = 1.87, 95% CI: 1.56 to 2.24), and log *E. coli* (OR = 2.01, 95% CI: 1.54 to 2.63). Using multivariable modeling, *Salmonella* detection in boneless beef was significantly associated with season, larger 325-g sample size (OR = 2.68, 95% CI: 2.41 to 2.99), increased log SPCs (OR = 1.15, 95% CI: 1.09 to 1.22), and increased log *E. coli* (OR = 1.65, 95% CI: 1.41 to

TABLE 3. *AMS vendor boneless beef indicator detection and* Salmonella *presence*

		Boneless beef					Ground beef				
			Outcome positive					Outcome positive			
Outcome	Indicator	No. of lots	*n*	%	OR	95% CI	No. of sublots	*n*	%	OR	95% CI
1 = *Salmonella* positive, 0 = *Salmonella* negative	SPCs										
	Detected	139,024	1,724	1.24	1.50	1.30–1.72	19,799	188	0.95	1.62	1.10–2.46
	Not detected	27,772	231	0.83			5,272	31	0.59		
	Total coliforms										
	Detected	13,829	210	1.52	1.34	1.15–1.55	3,205	63	1.97	2.79	2.04–3.77
	Not detected	152,967	1,745	1.14			21,846	156	0.71		
	E. coli										
	Detected	3,121	79	2.53	2.24	1.76–2.81	1,188	29	2.44	3.12	2.02–4.65
	Not detected	163,675	1,876	1.15			23,863	190	0.80		
1 = Dublin positive, 0 = other *Salmonella* serotype positive	SPCs										
	Detected	1,234	621	50.32	0.42	0.29–0.60	147	30	20.41	0.97	0.32–3.61
	Not detected	173	122	70.52			24	5	20.83		
	Total coliforms										
	Detected	153	35	22.88	0.23	0.15–0.34	46	5	10.87	0.39	0.11–1.11
	Not detected	1,254	708	56.46			125	30	24.00		
	E. coli										
	Detected	58	5	8.62	0.08	0.02–0.20	23	2	8.70	0.33	0.04–1.48
	Not detected	1,349	738	54.71			148	33	22.30		
1 = Dublin positive, 0 = *Salmonella* negative	SPCs										
	Detected	137,921	621	0.45	1.02	0.84–1.25	19,621	30	0.15	1.61	0.62–5.30
	Not detected	27,663	122	0.44			5,246	5	0.10		
	Total coliforms										
	Detected	13,654	35	0.26	0.55	0.38–0.77	3,147	5	0.16	1.15	0.35–3.00
	Not detected	151,930	708	0.47			21,720	30	0.14		
	E. coli										
	Detected	3,047	5	0.16	0.36	0.12–0.84	1,161	2	0.17	1.24	0.14–4.85
	Not detected	162,537	738	0.45			23,706	33	0.14		
1 = Other *Salmonella* serotype positive, 0 = *Salmonella* negative	SPCs										
	Detected	137,913	613	0.44	2.41	1.81–3.28	19,708	117	0.59	1.65	1.01–2.84
	Not detected	27,592	51	0.18			5,260	19	0.36		
	Total coliforms										
	Detected	13,737	118	0.86	2.40	1.95–2.94	3,183	41	1.29	2.97	2.01–4.35
	Not detected	151,768	546	0.36			21,785	95	0.44		
	E. coli										
	Detected	3,095	53	1.71	4.61	3.41–6.13	1,180	21	1.78	3.73	2.22–6.00
	Not detected	162,410	611	0.38			23,788	115	0.48		

TABLE 4. *General linear models for AMS boneless beef and* Salmonella *presence*[a]

Outcome	Variable	Boneless beef		Ground beef	
		OR	95% CI	OR	95% CI
1 = *Salmonella* positive, 0 = *Salmonella* negative	Sample size	2.68	2.41–2.99	1.55	1.12–2.14
	log SPCs	1.15	1.09–1.22	1.23	1.03–1.47
	log *E. coli*	1.65	1.41–1.94		
	Season				
	Summer	1.52	1.25–1.85	2.47	1.41–4.31
	Fall	2.79	2.35–3.32	1.56	0.90–2.71
	Winter	2.15	1.79–2.58	1.75	1.00–3.06
1 = *Salmonella* Dublin positive, 0 = other *Salmonella* serotype positive	Sample size	0.63	0.45–0.90	0.04	0.00–0.32
	log SPCs	0.63	0.52–0.76		
	log total coliforms	0.46	0.31–0.70		
	Season				
	Summer	0.16	0.08–0.32	0.25	0.02–2.87
	Fall	0.31	0.17–0.58	0.01	0.00–0.14
	Winter	0.16	0.08–0.30	0.08	0.01–0.87
1 = *Salmonella* Dublin positive, 0 = *Salmonella* negative	Sample size	3.95	3.32–4.71	0.16	0.05–0.58
	log SPCs	1.12	1.02–1.23		
	log total coliforms	0.68	0.50–0.91		
	Season				
	Summer	0.97	0.71–1.33	0.97	0.32–2.97
	Fall	2.62	2.04–3.37	0.04	0.01–0.22
	Winter	1.81	1.39–2.36	0.41	0.14–1.18
1 = Other *Salmonella* serotype positive, 0 = *Salmonella* negative	Sample size	2.22	1.84–2.66	2.12	1.41–3.19
	log SPCs	1.29	1.17–1.43	1.27	1.01–1.60
	log *E. coli*	1.89	1.57–2.29		
	Season				
	Summer	2.25	1.58–3.19	2.87	1.35–6.11
	Fall	2.54	1.82–3.56	2.61	1.26–5.43
	Winter	2.79	1.98–3.92	2.23	1.04–4.76

[a] Initial general linear models considered season, sample size, and concentrations of log SPCs, log total coliforms, and log *E. coli* with nesting by vendor. Spring was the seasonal referent.

1.94) (Table 4). In ground beef, *Salmonella* detection was associated with season, larger sample size (OR = 1.55, 95% CI: 1.12 to 2.14), and increased log SPC (OR = 1.23, 95% CI: 1.03 to 1.47).

Serotyping was done for 1,407 (72.0%) of 1,955 *Salmonella* isolates recovered from boneless beef lots (Table 5). Most of these isolates were *Salmonella* Dublin (n = 743, 52.8%), followed by *Salmonella* serotypes Newport (n = 147, 10.5%), Montevideo (n = 98, 7.0%), Anatum (n = 47, 3.3%), Typhimurium (n = 45, 3.2%), and Muenchen (n = 38, 2.7%). The proportion of serotyped *Salmonella* isolates determined to be *Salmonella* Dublin differed significantly in the univariable analysis by season ($\chi^2_3 = 42.5$, $P < 0.001$) and vendor ($\chi^2_{14} = 502.3$, $P < 0.001$). *Salmonella* Dublin was the most common serotype in all four seasons, accounting for 65.8% of serotypes in spring, 37.0% in summer, 58.7% in fall, and 49.3% in winter. Among vendors with at least 30 serotyped *Salmonella* isolates, the proportion of *Salmonella* Dublin isolates ranged from 88.7 to 1.6%. Unlike overall *Salmonella* prevalence, *Salmonella* Dublin prevalence among serotyped isolates did not differ significantly by pathogen sample size (OR = 1.06, 95% CI: 0.83 to 1.35).

In ground beef, serotypes were determined for 171 (78.4%) of 219 *Salmonella*-positive sublots (Table 5). The most common *Salmonella* serotype was *Salmonella* Dublin (n = 35, 20.5%), followed by *Salmonella* serotypes Montevideo (n = 27, 15.8%), Newport (n = 20, 11.7%), Anatum (n = 16, 9.4%), and Typhimurium (n = 11, 6.4%). The proportion of serotyped *Salmonella* isolates determined to be *Salmonella* Dublin differed significantly in the univariable analysis by season ($\chi^2_3 = 17.8$, $P < 0.001$) and vendor ($\chi^2_{10} = 52.2$, $P < 0.001$). *Salmonella* Dublin was the most common individual serotype found in each season except fall. *Salmonella* Dublin accounted for 43.8% of serotypes in spring, 24.4% in summer, and 28.3% in winter. It accounted for 3.5% of serotypes in fall. Among vendors with at least 15 serotyped *Salmonella* isolates, *Salmonella* Dublin prevalence ranged from 54.2 to 0% of isolates.

***Salmonella* Dublin compared with other *Salmonella* serotypes.** In boneless beef, *Salmonella* Dublin–positive lots (n = 743) were inversely associated with indicator microorganism concentrations compared with other *Salmonella* serotypes as a group (n = 664). Indicator microorganisms were less likely to be detected in *Salmonella*

TABLE 5. *AMS boneless beef* Salmonella *serotype performance*

	Boneless beef					Ground beef				
		Salmonella Dublin positives		Other *Salmonella* serotypes			*Salmonella* Dublin positives		Other *Salmonella* serotypes	
	No. of lots	*n*	%	*n*	%	No. of sublots	*n*	%	*n*	%
Overall	1,407	743	52.8	664	47.2	171	35	20.5	136	79.5
Season[a]										
Spring	120	79	65.8	41	34.2	16	7	43.8	9	56.3
Summer	235	87	37.0	148	63.0	45	11	24.4	34	75.6
Fall	618	363	58.7	255	41.3	57	2	3.5	55	96.5
Winter	434	214	49.3	220	50.7	53	15	28.3	38	71.7
Salmonella sample size[b]										
25 g	383	198	51.7	185	48.3	72	21	29.2	51	70.8
325 g	1,024	545	53.2	479	46.8	99	14	14.1	85	85.9

[a] Boneless beef: *Salmonella* Dublin, $\chi^2_3 = 42.5$, $P < 0.001$. Ground beef: *Salmonella* Dublin, $\chi^2_3 = 17.8$, $P < 0.001$.
[b] 325-g co-enriched sampling began 1 March 2015. Boneless beef: *Salmonella* Dublin, OR = 1.06, 95% CI: 0.83 to 1.35. Ground beef: *Salmonella* Dublin, OR = 0.40, 95% CI: 0.17 to 0.91.

Dublin–positive lots and, when detected, were present in lower concentrations compared with lots positive for other *Salmonella* serotypes. *Salmonella* Dublin–positive boneless beef lots were significantly less likely to contain detectable SPCs (OR = 0.42, 95% CI: 0.29 to 0.60), total coliforms (OR = 0.23, 95% CI: 0.15 to 0.34), and *E. coli* (OR = 0.08, 95% CI: 0.02 to 0.20) than lots that tested positive for other *Salmonella* serotypes (Table 3). Furthermore, these *Salmonella* Dublin–positive lots were significantly lower in log SPCs (OR = 0.63, 95% CI: 0.56 to 0.71), log total coliforms (OR = 0.29, 95% CI: 0.21 to 0.41), and log *E. coli* concentration (OR = 0.10, 95% CI: 0.04 to 0.25) using univariable analysis. A multivariable model determined *Salmonella* Dublin was significantly associated with season, pathogen sample size (OR = 0.63, 95% CI: 0.45 to 0.90), log SPCs (OR = 0.63, 95% CI: 0.52 to 0.76), and log total coliform concentration (OR = 0.46, 95% CI: 0.31 to 0.70) compared with other *Salmonella* serotypes (Table 4).

In ground beef, indicator microorganism detection and concentration rarely differed significantly between *Salmonella* Dublin and other *Salmonella* serotypes. There were no significant differences in indicator microorganism detection (SPCs, total coliforms, or *E. coli*) between *Salmonella* Dublin and other *Salmonella* serotypes (Table 3). However, *Salmonella* Dublin was associated with lower log SPC concentration compared with other *Salmonella* serotypes (OR = 0.71, 95% CI: 0.51 to 0.99). There were no similar associations with log coliform or log *E. coli* concentration. In the ground beef multivariable model, season and pathogen sample size were significantly associated with *Salmonella* Dublin, but indicator microorganism concentrations were not (Table 4). *Salmonella* Dublin was significantly less likely to be detected relative to the other *Salmonella* serotypes in ground beef using the larger pathogen sample size after March 2015 (OR = 0.04, 95% CI: 0.00 to 0.32).

***Salmonella* Dublin–positive lots compared with *Salmonella*-negative lots.** *Salmonella* Dublin–positive boneless beef lots were significantly less likely to contain detectable total coliforms (OR = 0.55, 95% CI: 0.38 to 0.77) or detectable *E. coli* (OR = 0.36, 95% CI: 0.12 to 0.84) compared with *Salmonella*-negative lots (Table 3). Using univariable analysis, lots with higher concentrations of log SPCs (OR = 0.92, 95% CI: 0.85 to 0.99), log coliforms (OR = 0.58, 95% CI: 0.43 to 0.77), and log *E. coli* (OR = 0.92, 95% CI: 0.85 to 0.99) were less likely to contain *Salmonella* Dublin than lots with lower indicator microorganism concentrations. In the boneless beef multivariable model, *Salmonella* Dublin was significantly associated with larger sample size (OR = 3.95, 95% CI: 3.32 to 4.71), higher log SPCs (OR = 1.12, 95% CI: 1.02 to 1.23), and lower log total coliform concentration (OR = 0.68, 95% CI: 0.50 to 0.91) compared with *Salmonella*-negative lots (Table 4).

In ground beef, however, there were no significant associations between *Salmonella* Dublin presence and indicator microorganism detection compared with the *Salmonella*-negative sublots. In univariable analysis, only log SPC concentration was significantly associated with *Salmonella* Dublin, which had a protective effect (OR = 0.71, 95% CI: 0.51 to 0.99) as concentration increased. In the multivariable model, *Salmonella* Dublin was significantly associated with season and sample size, but not with indicator organism concentrations. The multivariable model found *Salmonella* Dublin was significantly more likely in ground beef sublots sampled using the smaller, pre-March 2015 sampling protocol than in the larger, post-March 2015 samples (OR = 0.16, 95% CI = 0.05 to 0.58). Curiously, sample size had strong but opposite associations with *Salmonella* Dublin in boneless and ground beef. In boneless beef, *Salmonella* Dublin was significantly more likely to be present in the later and larger samples, but in ground beef, it was less likely to be present in the later and larger samples. *Salmonella* isolated from sublots produced after 1 March 2015 were less likely to be *Salmonella* Dublin (14.1%) than lots produced earlier (29.2%) (OR = 0.40, 95% CI: 0.17 to 0.91).

Other *Salmonella* serotypes compared with *Salmonella*-negative lots. Indicator microorganism detection was significantly associated with the detection of *Salmonella* serotypes other than *Salmonella* Dublin (Table 3). In boneless beef, non–*Salmonella* Dublin serotypes were significantly associated with SPC detection (OR = 2.41, 95% CI: 1.81 to 3.28), coliform detection (OR = 2.40, 95% CI: 1.95 to 2.94), and *E. coli* detection (OR = 4.61, 95% CI: 3.41 to 6.13). Under univariable analysis, boneless beef lots were significantly more likely to contain non–*Salmonella* Dublin serotypes as log SPCs (OR = 1.37, 95% CI: 1.26 to 1.49), log total coliforms (OR = 1.83, 95% CI: 1.61 to 2.08), and log *E. coli* (OR = 2.61, 95% CI: 2.19 to 3.10) increased. In the multivariable model, non–*Salmonella* Dublin serotypes were significantly associated with season, larger sample size (OR = 2.22, 95% CI: 1.84 to 2.66), higher log SPC concentration (OR = 1.29, 95% CI: 1.17 to 1.43), and higher log *E. coli* concentration (OR = 1.89, 95% CI: 1.57 to 2.29) (Table 4).

In ground beef, non–*Salmonella* Dublin serotypes were significantly associated with SPC detection (OR = 1.66, 95% CI: 1.01 to 2.84), total coliform detection (OR = 2.97, 95% CI: 2.01 to 4.35), and *E. coli* detection (OR = 3.73, 95% CI: 2.22 to 6.00). Univariable analysis found significant non–*Salmonella* Dublin associations with increased log SPCs (OR = 1.60, 95% CI: 1.34 to 1.91), log total coliforms (OR = 1.95, 95% CI: 1.56 to 2.44), and log *E. coli* concentration (OR = 2.27, 95% CI: 1.67 to 3.09). In the multivariable model, non–*Salmonella* Dublin serotypes were significantly associated with larger sample size (OR = 2.12, 95% CI: 1.41 to 3.19), higher log SPC concentration (OR = 1.27, 95% CI: 1.01 to 1.60), and season.

This study found consistent, positive associations between indicator detection and *Salmonella* presence and increased odds of *Salmonella* presence with increased concentration of log SPCs and log *E. coli* in boneless beef and increased log SPC concentration in ground beef. These findings support the continued use of microbial indicators as qualitative controls for boneless and ground beef production. Good hygiene and process control indicated by lower indicator microorganism concentrations appear to generally reduce the risk of *Salmonella* presence. However, the effectiveness of these measures was markedly reduced by the inclusion of lots contaminated by *Salmonella* Dublin. Generally, increased concentrations of log SPCs and log *E. coli* were associated with *Salmonella* overall and other *Salmonella* serotypes, but not with *Salmonella* Dublin presence. The differential associations of *Salmonella* Dublin presence relative to the other serotypes with microbial indicator concentration reduces their effectiveness to assess the risk of *Salmonella* presence. The results of this study provide new information that AMS-approved beef vendors for the National School Lunch Program can use to better understand testing results and further refine their food safety systems.

Compared with other serotypes, *Salmonella* Dublin was more likely in lots with nondetectable indicators and with lower log SPC concentrations. *Salmonella* Dublin also had a markedly different seasonality from that of other *Salmonella* serotypes. *Salmonella* Dublin was more strongly associated with spring compared with the other seasons. Spring had the highest proportion of *Salmonella* Dublin isolates in both boneless and ground beef. Furthermore, during spring, *Salmonella* Dublin prevalence tended to be significantly higher than during the other seasons.

Other studies have attributed higher *Salmonella* prevalence during the summer and fall to other environmental variables, including temperature, moisture, and latitude, that were not measured in this study *(10, 22)*. Although herd-level factors were not examined in this study, it is possible that the differential seasonal findings may also correlate with underlying cattle management practices within the source population. Pregnancy and calving-related stress in the spring may weaken a cow's immune system, thereby increasing the likelihood of latent infection reactivation and increased shedding by carriers *(19)*. This reactivation and shedding of *Salmonella* Dublin on farms may, in turn, contribute to higher *Salmonella* Dublin prevalence in meat processing operations. In months subsequent to spring, increased metabolic demand because of milk production may also affect *Salmonella* prevalence in herds and in meat processing. The observation that the proportion of *Salmonella* isolates found to be *Salmonella* Dublin decreased from 52.8% in boneless beef to 20.5% in ground beef was a key finding. Decreased *Salmonella* Dublin prevalence in ground beef may be because of variations in the growth phase among *Salmonella* serotypes, along with inherent variation in storage temperature and nutrient availability within lots and sublots during production and type of meat *(17, 23, 28, 29)*. *Salmonella* serotypes other than *Salmonella* Dublin may be outcompeting *Salmonella* Dublin during the period between boneless beef testing and ground beef testing on the basis of these and similar factors. These findings suggest *Salmonella* Dublin may be less fit than other *Salmonella* serotypes in boneless and ground beef containing higher microbial indicator concentrations. It is also possible that ground beef is a less hospitable environment for *Salmonella* Dublin for unknown reasons.

Future research should examine the risk of *Salmonella* contamination compared with various indicator microorganism concentration levels and combinations of those levels. It may be that certain combinations of indicator concentrations signal higher risk than other combinations. For example, a boneless beef lot containing higher concentrations of both log SPCs and *E. coli* (e.g., $<10^4$ CFU g ^{1}) may be more likely to contain *Salmonella* than a lot with relatively fewer SPCs, *E. coli,* or both. This research should be informed by the knowledge that risk may also vary by *Salmonella* serotype and by serotype prevalence in source cattle. Once identified, indicator concentration combinations that represent increased risk of *Salmonella* presence could serve as triggers for additional preventative actions aimed at reducing indicator concentration and thereby reducing risk of *Salmonella* presence in distributed beef products.

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