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Research Paper

Microbiological Testing Results of Boneless and Ground Beef Purchased for the U.S. National School Lunch Program, School Years 2015 to 2018

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ABSTRACT

The Agricultural Marketing Service (AMS) purchases beef for the National School Lunch Program and other federal nutrition assistance programs. For beef that will be delivered to food service facilities raw, each ca. 900-kg lot of boneless beef raw material and each ca. 4,500-kg sublot of resultant ground beef is tested for standard plate count (SPC) organisms, coliforms, *Escherichia coli, Salmonella,* and *E. coli* O157:H7. In addition, 1 of every 10 lots of boneless beef, randomly selected, is tested for *E. coli* O26, O45, O103, O111, O121, and O145. For beef that will be cooked using a validated lethality step at a federally inspected establishment before delivery, each lot of boneless beef and each sublot of ground beef is tested for SPC organisms, coliforms, and *E. coli* only. Any lot or sublot exceeding predefined critical limits (CLs) of 100,000 CFU g ^{1} for SPC organisms, 1,000 CFU g ^{1} for coliforms, or 500 CFU g ^{1} for *E. coli* or for beef containing *Salmonella* or any of previously mentioned *E. coli* serotypes is rejected for purchase. For school years 2015 through 2018 (July 2014 through June 2018), 220,497,254 kg of boneless beef and 189,347,318 kg of ground beef were produced for AMS. For boneless beef, 133 (0.06%), 164 (0.07%), and 106 (0.04%) of 240,488 lots exceeded CLs for SPC organisms, coliforms, and *E. coli,* respectively; 2,038 (1.30%) and 116 (0.07%) of 156,671 lots were positive for *Salmonella* and *E. coli* O157:H7, respectively; and 59 (0.36%) of 16,515 lots were positive for non-O157 Shiga toxin–producing *E. coli*. For ground beef, 46 (0.10%), 27 (0.06%), and 19 (0.04%) of 45,769 sublots exceeded CLs for SPC organisms, coliforms, and *E. coli,* respectively; and 329 (1.40%) and 18 (0.08%) of 23,475 sublots were positive for *Salmonella* and *E. coli* O157:H7, respectively. All lots and sublots found to exceed indicator organism CLs or to contain pathogens were identified, rejected for purchase, and diverted from federal nutrition assistance programs.

HIGHLIGHTS

- AMS purchases beef for the National School Lunch Program.
- Less than 0.10% of beef samples exceeded indicator organism critical limits.
- *Salmonella* was found in 1.4% and *E. coli* O157:H7 was found in 0.08% of samples.
- Indicator critical limit exceedance was weakly associated with pathogen presence.
- Beef with excessive indicator organisms or containing pathogens was rejected for purchase.

Key words: Beef; *Escherichia coli* O157:H7; National School Lunch Program; *Salmonella*

The Agricultural Marketing Service (AMS) purchases food for federal nutrition assistance programs. Prominent among these, the National School Lunch Program (NSLP) provides food to approximately 31 million schoolchildren in more than 100,000 participating institutions *(26)*. Boneless and ground beef are staples of the NSLP, with average purchases of 46.7 million kg per year for 2015 through 2018 *(1)*.

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AMS purchase specifications for boneless and ground beef include requirements for domestic origin, harvest (slaughter), quality control, and animal handling and welfare *(3)*. Food safety requirements are also included in the purchase specifications. These include that beef must be produced under a grant of federal inspection and that harvest establishments identify and implement at least two pathogen intervention steps, one of which must be scientifically validated to achieve a 3-log reduction of enteric pathogens.

AMS also tests boneless and ground beef it intends to purchase for various microorganisms *(3)*. For raw beef

scheduled to be delivered directly to schools, every approximately 900 kg of boneless beef used as raw material and every 4,500 kg of ground beef finished product are tested for the presence of *Escherichia coli* O157:H7 and *Salmonella* and for concentrations of standard plate count (SPC) organisms, coliforms, and *E. coli*. Since June 2014, 1 of every 10 lots of boneless beef, randomly selected, has been tested for non-O157 Shiga toxin–producing *E. coli* (STEC; *E. coli* O26, O45, O103, O111, O121, and O145).

For boneless beef and coarse ground beef raw materials scheduled to be cooked using a validated lethality step at a federally inspected establishment and then delivered to schools, each lot is tested for concentrations of SPC organisms, coliforms, and *E. coli* only. Lots positive for *E. coli* O157:H7, non-O157 STEC, or *Salmonella* or with concentrations exceeding predefined critical limits (CLs) of indicator microorganisms are rejected for purchase and diverted from federal nutrition assistance programs.

To keep stakeholders informed, data generated from AMS testing of boneless and ground beef are summarized quarterly and posted to the AMS Web site *(2)*. More detailed analyses are done throughout the year to better understand the findings, identify causes of contamination, help guide the development and implementation of corrective actions, and revise AMS purchase program requirements. Data for school years 2011 through 2014 were analyzed and described previously *(13)*. Here, we describe data for school years 2015 through 2018 (July 2014 through June 2018).

MATERIALS AND METHODS

Sample collection. All samples were collected by trained employees of AMS vendor establishments *(4)*. For boneless beef, samples representing the beef carcass exterior were collected using one of two sampling methods from each approximately 900-kg lot by AMS vendor establishment employees. The two sampling method options were the N60 excision procedure *(28)* and the N60 Plus modified excision mechanical sampling procedure *(21)*. Establishments using the latter method provided in-house validation study documentation, indicating it was as effective as the excision method at recovering comparable microbial loads in boneless beef trimmings. For daily production lots of ground beef, grab samples were collected randomly for each approximately 4,500-kg sublot.

Microbiological analyses were done according to the Food Safety and Inspection Service (FSIS) *Microbiology Laboratory Guidebook (28)*. In 2014, FSIS increased the sample size of boneless and ground beef for *Salmonella* testing from 25 to 325 g. However, AMS did not require vendors to collect the larger sample size until March 2015. Therefore, an 8-month aggregate of data within school year 2015, from July 2014 to March 2015, is affected. Until March 2015, a 325-g ± 10% (293- to 358-g) sample was collected and used in the assay to detect *E. coli* O157:H7 and a separate 25-g ± 10% (2.5-g) sample was collected and used in the assay to detect *Salmonella*. Beginning in March 2015, a single 325-g ± 10% (293- to 358-g) sample was collected and used in the assay to detect *E. coli* O157:H7 and *Salmonella*. For boneless beef only, this sample was also used for 1 of every 10 lots, randomly selected by the laboratory, for testing for non-O157 STEC. A separate 25-g ± 10% (23- to 28-g) sample was collected and used in the assay to quantify SPC organisms, coliforms, and *E. coli* for both boneless and ground beef. In all cases, fresh (not frozen) samples were collected and aseptically transferred into Whirl-Pak bags, sealed, and placed on prefrozen gel ice packs in insulated shipping containers.

All samples collected by AMS vendor establishment employees were sent to an AMS-designated laboratory (ADL). Establishment employees completed sample submission forms (which included tracking numbers, weight of product samples, lot numbers, and similar information) supplied by the ADL. Sample submission forms were placed with the samples inside the insulated shipping containers and sent overnight to the ADL.

Sample receipt and processing. Sample receipt and processing was done as previously described *(13)*. *Salmonella* isolates recovered through the AMS testing program were sent by AMS ADLs to the FSIS Eastern Laboratory in Athens, Georgia, where they were serotyped using the method described by McQuiston et al. *(19)* and tested for antimicrobial susceptibility using the methods and interpretive criteria of the National Antimicrobial Resistance Monitoring System (NARMS) *(20)*. The following antimicrobials and resistance breakpoints (in micrograms per milliliter) were included: gentamicin (16), kanamycin (64), streptomycin (32), amoxicillin–clavulanic acid (32/16), meropenem (4), cefoxitin (32), ceftriaxone (4), sulfamethoxazole or sulfisoxazole (512), trimethoprim-sulfamethoxazole (4/76), azithromycin (32), ampicillin (32), chloramphenicol (32), ciprofloxacin (1), nalidixic acid (32), and tetracycline (16).

For detecting and quantifying SPC organisms, total coliforms, and *E. coli* in boneless beef, protocols described in Section 3.2 of the FSIS *Microbiology Laboratory Guidebook (28)* were used. A 25-g sample was placed into a sterile blender jar or stomacher bag, 450 mL of sterile Butterfield's phosphate diluent or buffered peptone water was added, and the sample was blended or shaken vigorously for 2 min. The resultant homogenate was serially diluted. SPC organisms were quantified using the Petrifilm method described in Section 3.6.2 of the FSIS *Microbiology Laboratory Guidebook (28)*. Total coliforms and *E. coli* were quantified using the method described in Section 3.7.2 of the FSIS *Microbiology Laboratory Guidebook (28)*.

Result reporting. Microbiological results were provided by the ADL to the AMS in comma-separated value files uploaded to a Microsoft SQL database. Serotype information was provided quarterly by electronic mail from FSIS to AMS and manually input into Microsoft Excel spreadsheets.

Data analysis. Microbiological test results were organized and summarized using Microsoft Excel pivot table functions. Tibco Spotfire Desktop 7.13.0 was used to examine the data for relationships between pathogen-positive samples and those in which indicator microorganisms exceeded CLs. Odds ratios (ORs) were calculated using Stata 14.2.

The Centers for Disease Control and Prevention (CDC) National Outbreak Reporting System (NORS) was queried for *Salmonella* and *E. coli* outbreaks in school settings between calendar years 2010 and 2017 *(10)*. Outbreak vehicle and type of school setting were determined to identify any outbreaks associated with ground beef obtained through the NSLP.

RESULTS

During school years 2015 through 2018, 220,497,254 kg of boneless beef was tested (Table 1). The boneless beef

TABLE 1. *AMS boneless beef indicator microorganism performance by school year*

School year[a]	Total kg, no.	Lots, no. (%)	SPC critical limit exceeded, no. (%)[b]	Coliform critical limit exceeded, no. (%)[c]	*E. coli* critical limit exceeded, no. (%)[d]
2015	50,258,723	54,284 (22.6)	18 (0.03)	38 (0.07)	24 (0.04)
2016	55,751,935	60,801 (25.3)	32 (0.05)	40 (0.07)	27 (0.04)
2017	58,641,394	64,289 (26.7)	52 (0.08)	34 (0.05)	24 (0.04)
2018	55,845,202	61,114 (25.4)	31 (0.05)	52 (0.09)	31 (0.05)
Total	220,497,254	240,488 (100.0)	133 (0.06)	164 (0.07)	106 (0.04)

[a] July through June.
[b] Standard plate count (SPC) critical limit: 100,000 CFU g ^{1}.
[c] Total coliform critical limit: 1,000 CFU g ^{1}.
[d] *E. coli* critical limit: 500 CFU g ^{1}.

was tested in 240,488 lots (an average of 917 kg per lot). Testing showed 133 lots (0.06%) exceeded the SPC CL, 164 lots (0.07%) exceeded the coliform CL, and 106 lots (0.04%) exceeded the *E. coli* CL.

Of the 240,488 boneless beef lots, 156,671 (65.15%) were scheduled for use as raw materials for uncooked ground beef delivery to federal nutritional assistance programs and were therefore tested for *E. coli* O157:H7, *Salmonella,* and non-O157 STEC (Table 2). Testing showed 116 lots (0.07%) were positive for *E. coli* O157:H7, and 2,038 lots (1.30%) tested positive for *Salmonella*. In addition, 59 (0.36%) of 16,515 randomly selected boneless beef lots tested positive for non-O157:H7 STEC (Table 3).

Lots produced from school year 2016 onward and that exclusively used the larger 325-g coenriched pathogen sample were significantly more likely to test positive for *Salmonella* than lots produced in school year 2015 (OR = 1.55, 95% confidence interval [95% CI] = 1.39 to 1.73). In contrast, lots produced from school year 2016 onward were significantly less likely to test positive for *E. coli* O157:H7 compared with previous years (OR = 0.35, 95% CI = 0.24 to 0.51).

For the same school years, 189,347,318 kg of ground beef was tested for indicator microorganisms (Table 4). The ground beef was tested in 45,769 sublots (an average of 4,137 kg per sublot). Testing showed that 46 sublots (0.10%) exceeded the SPC CL, 27 sublots (0.06%) exceeded the coliform CL, and 19 sublots (0.04%) exceeded the *E. coli* CL. Of the 45,769 sublots, 23,475 (51.29%) were scheduled for raw, uncooked delivery to federal nutrition assistance programs and thus tested for *E. coli* O157:H7 and *Salmonella* (Table 5). Of these sublots, 18 (0.08%) tested positive for *E. coli* O157:H7 and 329 (1.40%) tested positive for *Salmonella*. Sublots of ground beef produced between school years 2016 and 2018 were significantly more likely to test positive for *Salmonella* than lots produced in school year 2015 (OR = 2.28, 95% CI = 1.75 to 3.00). There was no significant difference in *E. coli* O157:H7–positive samples in school year 2015 compared with subsequent years (OR = 1.31, 95% CI = 0.45 to 4.25).

Pathogen prevalence in ground beef was compared with its prevalence in source boneless beef. *Salmonella* prevalence was higher in ground beef (1.40%) compared with boneless beef (1.30%), but the difference was not statistically significant (OR = 1.08, 95% CI = 0.96 to 1.21). Similarly, *E. coli* O157:H7 prevalence was higher in ground beef (0.08%) than boneless beef (0.07%), but the difference was not statistically significant (OR = 1.04, 95% CI = 0.59 to 1.71).

Associations between CL exceedance and pathogen presence were weak. Of the 95 boneless beef lots that exceeded the SPC CL, 2 (2.11%) were *Salmonella* positive. Of the 164 lots that exceeded the coliform CL, 3 (1.83%) were *Salmonella* positive. Of the 106 lots that exceeded the *E. coli* CL, 3 (2.83%) were *Salmonella* positive. Exceeding CLs was not associated with significantly increased *Salmonella* presence for SPC organisms (OR = 1.63, 95% CI = 0.19 to 6.07), coliforms (OR = 1.41, 95% CI = 0.29 to

TABLE 2. *AMS boneless beef* E. coli *O157:H7 and* Salmonella *performance by school year*

School year[a]	Total kg, no.	Lots, no. (%)	*E. coli* O157:H7 positive, no. (%)[b,c]	*Salmonella* positive, no. (%)[b,d]
2015[e]	42,508,987	45,849 (29.3)	63 (0.14)	431 (0.94)
2016	47,766,214	52,021 (33.2)	18 (0.03)	931 (1.79)
2017	27,398,382	29,887 (19.1)	15 (0.05)	394 (1.32)
2018	26,662,635	28,914 (18.5)	20 (0.07)	282 (0.98)
Total	144,336,217	156,671 (100.0)	116 (0.07)	2,038 (1.30)

[a] July through June.
[b] *E. coli* O157:H7 and *Salmonella* critical limit: positive (+) result 325 g ^{1}.
[c] Odds of *E. coli* O157:H7 presence from school years 2016 through 2018 compared with school year 2015: OR = 0.35, 95% CI = 0.24 to 0.51.
[d] Odds of *Salmonella* presence from school years 2016 through 2018 compared with school year: OR = 1.55, 95% CI = 1.39 to 1.73.
[e] July 2014 to February 2015; *Salmonella* critical limit: positive (+) result, 25 g ^{1}.

TABLE 3. *AMS boneless beef non-O157 STEC performance by school year*

School year[a]	Total kg, no.	Lots, no. (%)	Non-O157 STEC positive, no. (%)[b,c]
2015	4,500,603	4,692 (28.4)	19 (0.40)
2016	5,099,809	5,562 (33.7)	30 (0.54)
2017	2,957,906	3,231 (19.6)	6 (0.19)
2018	2,802,325	3,030 (18.4)	4 (0.13)
Total	15,360,643	16,515 (100.0)	59 (0.36)

[a] July through June.
[b] *E. coli* serotypes O26, O45, O103, O111, O121, and O145.
[c] Non-O157 STEC critical limit: positive (+) result, 325 g ^{1}.

4.21), or *E. coli* (OR = 2.21, 95% CI = 0.45 to 6.65). Pearson correlations between SPC, coliforms, and *E. coli* with *Salmonella* were low (r = 0.145, 0.135, and 0.168, respectively). No boneless beef lot that exceeded an indicator CL was positive for *E. coli* O157:H7.

For ground beef, of 25 sublots that exceeded the SPC CL, 1 (4%) was positive for *Salmonella*. Exceeding the SPC CL was not significantly associated with *Salmonella* presence (OR = 2.94 95% CI = 0.07 to 18.12). Pearson correlation between SPC CL exceedance and *Salmonella* in ground beef was 0.199. Of the 15 sublots that exceeded the coliform CL, none tested positive for *Salmonella* or *E. coli* O157:H7. Of the 9 sublots that exceeded the *E. coli* CL, none tested positive for *Salmonella* or *E. coli* O157:H7.

A total of 1,309 *Salmonella* isolates from 2,038 boneless beef lots were serotyped and tested for antimicrobial susceptibility. *Salmonella* Dublin was the most frequent serotype (n = 681, 52.0%), followed by *Salmonella* Newport (n = 141, 10.8%) and *Salmonella* Montevideo (n = 105, 8.0%; Table 6). In addition, 475 isolates (36.3%) were susceptible to all antimicrobials on the panel (pansusceptible), 35 isolates (2.7%) were resistant to no more than two antimicrobials, and 799 isolates (61.0%) were resistant to at least three antimicrobials (Table 7).

In ground beef, *Salmonella* isolates from 251 sublots were serotyped and tested for antimicrobial susceptibility. *Salmonella* Montevideo was the most frequent serotype (n = 85, 33.9%), followed by *Salmonella* Dublin (n = 35, 13.9%) and *Salmonella* Newport (n = 24, 9.6%; Table 8). In addition, 173 (68.9%) ground beef isolates were pansusceptible, 13 (5.2%) were resistant to no more than two antimicrobials, and 65 (25.9%) were resistant at least three antimicrobials (Table 9). *Salmonella* isolates recovered from ground beef were significantly more likely to be pansusceptible (OR = 3.89, 95% CI = 2.89 to 5.27) and significantly less likely to be resistant to at least three antimicrobials (OR = 0.22, 95% CI = 0.16 to 0.30) than those recovered from boneless beef.

TABLE 5. *AMS ground beef* E. coli *O157:H7 and* Salmonella *performance by school year*

School year[a]	Total kg, no.	Sublots, no. (%)	*E. coli* O157:H7 positive, no. (%)[b,c]	*Salmonella* positive, no. (%)[d]
2015[e]	38,855,723	9,287 (39.6)	6 (0.06)	74 (0.80)
2016	18,042,628	4,236 (18.0)	7 (0.17)	48 (1.13)
2017	20,742,450	4,892 (20.8)	3 (0.06)	56 (1.14)
2018	19,737,271	5,060 (21.6)	2 (0.04)	151 (2.98)
Total	97,378,072	23,475 (100.0)	18 (0.08)	329 (1.40)

[a] July through June.
[b] *E. coli* O157:H7 and *Salmonella* critical limit: positive (+) result, 325 g ^{1}.
[c] Odds of *E. coli* O157:H7 presence from school years 2016 through 2018 compared with school year 2015: OR = 1.31, 95% CI = 0.45 to 4.25.
[d] Odds of *Salmonella* presence from school years 2016 through 2018 compared with school year 2015: OR = 2.28, 95% CI = 1.75 to 3.00.
[e] July 2014 to February 2015, *Salmonella* critical limit: positive (+) result, 25 g ^{1}.

For school years 2015 through 2018, the six most frequently reported *Salmonella* serotypes in boneless and ground beef were identical. These were *Salmonella* serotypes Dublin, Newport, Montevideo, Typhimurium, Muenchen, and Anatum. While boneless and ground beef each had the same six most frequent serotypes, the order of the six was different. *Salmonella* Dublin composed 52.0% of isolates in boneless beef compared with 13.9% of isolates in boneless beef (OR = 6.69, 95% CI = 4.58 to 10.01). In contrast, *Salmonella* Montevideo composed 8.0% of isolates in boneless beef and 33.9% of isolates in ground beef (OR = 0.17, 95% CI = 0.12 to 0.24). The prevalence of *Salmonella* Dublin detection in boneless and ground beef was 43.5 of 10,000 lots and 14.9 of 10,000 sublots, respectively. The prevalence of *Salmonella* Montevideo

TABLE 4. *AMS ground beef indicator organism performance by school year*

School year[a]	Total kg, no.	Sublots, no. (%)	SPC critical limit exceeded, no. (%)[b]	Coliform critical limit exceeded, no. (%)[c]	*E. coli* critical limit exceeded, no. (%)[d]
2015	39,552,867	9,453 (20.7)	24 (0.25)	3 (0.03)	3 (0.03)
2016	47,401,073	11,216 (24.5)	11 (0.10)	4 (0.04)	9 (0.08)
2017	52,869,100	12,713 (27.8)	4 (0.03)	13 (0.10)	4 (0.03)
2018	49,524,277	12,387 (27.1)	7 (0.06)	7 (0.06)	3 (0.02)
Total	189,347,318	45,769 (100.0)	46 (0.10)	27 (0.06)	19 (0.04)

[a] July through June.
[b] Standard plate count (SPC) critical limit: 100,000 CFU g ^{1}.
[c] Total coliform critical limit: 1,000 CFU g ^{1}.
[d] *E. coli* critical limit: 500 CFU g ^{1}.

TABLE 6. Salmonella *serotypes recovered from boneless beef during school years 2015 to 2018*[a]

Salmonella serotype	No. (%) of isolates
Dublin	681 (52.02)
Newport	141 (10.77)
Montevideo	105 (8.02)
Typhimurium	43 (3.28)
Muenchen	36 (2.75)
Anatum	31 (2.37)
Give	27 (2.06)
Cerro	22 (1.68)
Muenster	22 (1.68)
Kentucky	15 (1.15)
Agona	14 (1.07)
Infantis	12 (0.92)
Altona	11 (0.84)
Meleagridis	10 (0.76)
Cubana	9 (0.69)
Norwich	9 (0.69)
Oranienburg	8 (0.61)
I4,5,12:i:	7 (0.53)
Thompson	7 (0.53)
Uganda	7 (0.53)
Barranquilla	6 (0.46)
Bredeney	6 (0.46)
Mbandaka	6 (0.46)
Schwarzengrund	6 (0.46)
Enteritidis	5 (0.38)
Minnesota	5 (0.38)
Senftenberg	4 (0.31)

[a] $n = 1{,}309$. In addition to the serotypes shown in the table, three isolates were recovered of Albany, Brandenburg, Kiambu, Poona, Rissen, Rough O:z4,z23: , and Sandiego; two isolates were recovered of 6,14,25:a:1,7, Braenderup, Falkensee, Havana, Manhatta, and Memphis; and one isolate was recovered of 13,23: :l,w, 3,10: :l,w, 6,7:Nonmotile, Adelaide, Berta, Blockley, Choleraesuis, Derby, Johannesburg, Lille, Livingstone, Orion_var_15+, Orion_var_15+,3, Oslo, Panama, Reading, Rough_O:k:1,5, Rough_O:gms: , Saintpaul, Soerenga, and Tennessee.

TABLE 7. Salmonella *antimicrobial resistance profiles recovered from boneless beef during school years 2015 to 2018*[a]

Resistance profile	No. (%) of isolates
Pansusceptible	475 (36.29)
Aug Amp Fox Tio Axo Chl Str Fis Tet	232 (17.72)
Aug Amp Fox Tio Axo Chl Nal Str Fis Tet	115 (8.79)
Aug Amp Fox Axo Chl Str Fis Tet	114 (8.71)
Chl Fis Str Tet	55 (4.20)
Aug Amp Fox Axo Chl Nal Str Fis Tet	48 (3.67)
Chl Nal Fis Str Tet	26 (1.99)
Aug Amp Fox Tio Axo Chl Gen Fis Tet	23 (1.76)
Aug Amp Tio Axo Chl Str Fis Tet	19 (1.45)
Aug Amp Fox Tio Axo Chl Str Fis Tet Cot	18 (1.38)
Amp Str Fis Tet	15 (1.15)
Tet	14 (1.07)
Aug Amp Axo Chl Nal Str Fis Tet	12 (0.92)
Nal Str Fis Tet	11 (0.84)
Aug Amp Chl Nal Str Fis Tet	10 (0.76)
Aug Amp Axo Chl Str Fis Tet	9 (0.69)
Aug Amp Fox Axo ChlStr Fis Tet Cot	9 (0.69)
Aug Amp Chl Str Fis Tet	8 (0.61)
Aug Amp Tio Axo Chl Nal Str Fis Tet	8 (0.61)
Fis	5 (0.38)
Str Tet	5 (0.38)
Amp Chl Str Fis Tet	4 (0.31)
Aug Amp Fox Tio Axo Str Fis Tet	4 (0.31)
Fis Tet	4 (0.31)
Str Fis Tet	4 (0.31)
Aug Amp Fox Tio Axo Chl Gen Fis	3 (0.23)
Aug Aump Fox Tio Axo Chl Nal Str Fis Tet Cot	3 (0.23)
Aug Amp Fox Tio Axo Chl Str Tet	3 (0.23)
Chl Str Fis Tet Cot	3 (0.23)

[a] $n = 1{,}309$. Amp, ampicillin; Aug, amoxicillin–clavulanic acid; Axo, ceftriaxone; Chl, chloramphenicol; Cot, trimethoprim-sulfamethoxazole; Fis, sulfamethoxazole-sulfisoxazole; Fox, cefoxitin; Gen, gentamicin; Nal, nalidixic; Str, streptomycin; Tet, tetracycline; Tio, ceftiofur. In addition to those shown in the table, two isolates were recovered for each of 14 separate antimicrobial resistance profiles and one isolate was recovered for each of 25 separate antimicrobial resistance profiles.

detection in boneless and ground beef was 6.7 of 10,000 lots and 36.2 of 10,000 sublots, respectively. *Salmonella* Dublin isolates were frequently antimicrobial resistant in both boneless and ground beef. Among *Salmonella* Dublin isolates from boneless beef lots and ground beef sublots, 95.7 and 100%, respectively, displayed resistance to at least three antimicrobials. The reduction in *Salmonella* Dublin prevalence from boneless to ground beef led to the overall reduction of antimicrobial-resistant *Salmonella* in ground beef compared with boneless beef.

Twenty-nine outbreaks of *Salmonella* and STEC infections in U.S. schools, colleges, and universities between 2010 and 2017 were reported to the CDC through NORS: 19 outbreaks of *Salmonella* and 10 outbreaks of STEC. Twelve outbreaks occurred in schools serving kindergarten through 12th grade (eight *Salmonella* and four STEC). None of these outbreaks were associated with beef products procured by AMS for the NSLP.

DISCUSSION

In procuring ground beef, AMS requires microbiological testing at two critical points in the production continuum: after harvesting when carcasses are converted into boneless beef trim and before final packaging of ground product. Concentrations of indicator microorganisms are used as measures of overall process control, and dispositional testing for pathogens is done to monitor the safety of beef that will be delivered raw.

Indicator microorganisms are helpful for examining the hygiene of beef carcasses and their resultant products and for evaluating the effectiveness of processing aids and interventions *(8, 23)*. The observation that less than 0.10% of boneless beef lots produced for AMS in school years 2015 through 2018 exceeded CLs for SPC organisms, coliforms, or *E. coli,* suggests the beef was produced under stringent sanitary controls. Compared with boneless beef produced for AMS during school years 2011 through 2014 *(13),* boneless beef produced for AMS during school years

TABLE 8. Salmonella *serotypes recovered from ground beef during school years 2015 to 2018*[a]

Salmonella serotype	No. (%) of isolates
Montevideo	85 (33.86)
Dublin	35 (13.94)
Newport	24 (9.56)
Anatum	15 (5.98)
Typhimurium	13 (5.18)
Muenchen	10 (3.98)
Meleagridis	8 (3.19)
Give	6 (2.39)
I4,5,12:i:	6 (2.39)
Cerro	5 (1.99)
Mbandaka	5 (1.99)
Norwich	5 (1.99)
Agona	4 (1.59)
Hadar	3 (1.20)
Muenster	3 (1.20)
Derby	2 (0.80)
Johannesburg	2 (0.80)
Reading	2 (0.80)
Sandiego	2 (0.80)
Senftenberg	2 (0.80)

[a] $n = 251$. In addition to the serotypes shown in the table, one isolate of each of the following serotypes was recovered: 13,23: :l,w, 3,10: :l,w, 6,7:Nonmotile, Adelaide, Berta, Blockley, Choleraesuis, Derby, Johannesburg, Lille, Livingstone, Orion_Var_15+, Orion_Var_15+,3. . . , Oslo, Panama, Reading, Rough O:k:1,5, Rough O:gms: , Saintpaul, Soerenga, and Tennessee.

TABLE 9. Salmonella *antimicrobial resistance profiles recovered from ground beef during school years 2015 to 2018*[a]

Resistance profile	No. (%) of isolates
Pansusceptible	173 (68.92)
Aug Amp Fox Axo Chl Str Fis Tet	22 (8.76)
Aug Amp Fox Tio Axo Chl Nal Str Fis Tet	11 (4.38)
Aug Amp Fox Tio Axo Chl Str Fis Tet	11 (4.38)
Aug Amp Fox Axo Chl Nal Str Fis Tet	6 (2.39)
Tet	5 (1.99)
Str Tet	4 (1.59)
Amp Str Fis Tet	3 (1.20)
Chl Str Fis Tet	3 (1.20)
Amp Chl Str Fis Tet	2 (0.80)
Str Fis	2 (0.80)

[a] $n = 251$. Amp, ampicillin; Aug, amoxicillin–clavulanic acid; Axo, ceftriaxone; Chl, chloramphenicol; Cot, trimethoprim-sulfamethoxazole; Fis, sulfamethoxazole-sulfisoxazole; Fox, cefoxitin; Gen, gentamicin; Nal, nalidixic; Str, streptomycin; Tet, tetracycline; Tio, ceftiofur. In addition to those shown in the table, one isolate was recovered for each of nine separate antimicrobial resistance profiles.

2015 through 2018 was significantly less likely to have SPC concentrations exceeding the CL (0.06% of samples from 2015 through 2018 compared with 0.10% of samples from 2011 through 2014) but significantly more likely to have *E. coli* concentrations exceeding the CL (0.04% of samples from 2015 through 2018 compared with 0.03% of samples collected from 2011 to 2014). The reasons for this are not clear. AMS will continue to closely monitor indicator organism performance of boneless beef and partner with vendors to help ensure process control is maintained.

Pathogen testing helps detect, and subsequently remove from the AMS procurement program, pathogen-positive product. Slightly more than 1% of the boneless beef lots produced for AMS for school years 2015 through 2018 were found to be positive for *Salmonella,* and less than 0.50% of the lots were found to be positive for *E. coli* O157:H7 and other STEC serotypes. The low level of pathogens found in boneless beef produced for AMS likely results from the multipronged approach to food safety used by the beef processing industry *(32)*. That AMS was able to identify these pathogen-positive lots and remove them from production resulted in decreased incidence of *Salmonella* and *E. coli* O157:H7 in ground beef compared with what it would have otherwise been. The increase in *Salmonella*-positive boneless beef lots is likely due to the sampling change mid-school year 2015, in which sample size was increased from 25 to 325 g. Before implementation, FSIS projected that the *Salmonella* sample size change would result in an increase in the number of *Salmonella*-positive samples *(27)*. Data from this study suggest the prediction was accurate.

Observations made for ground beef produced for school years 2015 through 2018 were like those made for boneless beef during the same period. Less than 0.10% of the lots of ground beef produced for AMS contained concentrations of indicator organisms exceeding CLs, again demonstrating the strong process control maintained by AMS vendors. *Salmonella*-positive ground beef sublots, although found at an incidence of under 1.50%, were more likely to occur for school years 2015 through 2018 compared with school years 2011 through 2014 (1.40% compared with 0.77%) *(13)*. As with boneless beef, this is likely because of the sampling protocol change mid-school year 2015, in which sample size was increased from 25 to 325 g. However, ground beef sampling size for *E. coli* O157:H7 remained constant at 325 g throughout school years 2011 through 2018; thus, sampling size variation does not explain the observation that *E. coli* O157:H7–positive lots of boneless beef were more likely to occur for school years 2015 through 2018 compared with school years 2011 through 2014. The increase in *E. coli* O157:H7–positive lots, although unexplained, highlights the need for continued investigation into methods for modeling pathogenic *E. coli* distribution during grinding *(17, 18)* and developing mitigations to further limit it *(25, 34, 35)*.

Salmonella Dublin, a host-adapted serotype found in both symptomatic and asymptomatic North American dairy cattle *(12, 16, 29, 31),* was the most commonly detected *Salmonella* serotype in boneless beef produced for school years 2015 through 2018. Its underlying prevalence in source herds is likely a key factor in its occurrence in boneless beef produced for AMS. On-farm mitigations, including purchasing animals from *Salmonella* Dublin–negative herds and good pen management, appear effective

in limiting *Salmonella* Dublin *(22)*. More research is needed to define additional mitigation strategies.

Salmonella Montevideo was the most commonly detected *Salmonella* serotype in ground beef produced for AMS. *Salmonella* Montevideo occurs on dairy farms *(9)* and thus may be introduced into beef during slaughter and processing. In addition, *Salmonella* Montevideo has been shown to colonize cattle lymph nodes *(21),* and although AMS requires removal of major lymph glands from boneless beef before grinding, it is possible that grinding of smaller lymph nodes helps explain the increased incidence of *Salmonella* Montevideo in ground beef procured by AMS.

Approximately 65% of *Salmonella* isolates from boneless beef and approximately 30% of those from ground beef were resistant to one or more antimicrobials. Extended-spectrum cephalosporins are important antimicrobials for treating salmonellosis in children. The observation of ceftriaxone and/or cefoxitin resistance among the *Salmonella* isolates recovered in this study is thus concerning. Neither ceftriaxone nor cefoxitin is approved for therapeutic use in food animals, but a closely related cephalosporin, ceftiofur, has been approved *(30)*. Ceftiofur use may thus help explain the occurrence of ceftiofur-ceftriaxone-cefoxitin coresistant *Salmonella* isolates in boneless and ground beef produced for AMS.

Data from this study suggest some additional general conclusions about the AMS boneless and ground beef purchase program and are helpful for guiding future research. First, a key factor likely behind the relatively low incidence of *Salmonella*-positive boneless beef lots and ground beef sublots is the AMS purchase requirement that major lymph glands be removed from boneless beef *(3)*. *Salmonella* is found in the lymph nodes of asymptomatic cattle *(24),* where they may persist for up to a month *(14)*. Thus, removing lymphatic material from boneless beef before grinding is a key mitigation in the AMS purchase program. As the impact of cattle type, location, and season on *Salmonella* carriage in bovine lymph nodes is elucidated *(5, 7, 21, 33),* beef producers and processors will likely be able to further mitigate the occurrence of *Salmonella* in boneless and ground beef. The efficacy of and feasibility for implementing *Salmonella* vaccination programs in cattle *(11, 15)* should continue to be explored.

Second, indicator organism CL exceedance was not significantly correlated with pathogen presence. Although past research suggests a lack of correlation between indicator organisms and pathogen presence *(23),* a study of pig slaughtering in Belgium found small but significant associations between *Enterobacteriaceae* concentrations and *Salmonella* presence *(6)*. However, our study examined correlation between CL exceedance and pathogen presence and not, more broadly, a continuum of indicator concentration and pathogen presence. The risk of pathogen presence within the AMS data sets may also vary by indicator concentration. We are exploring relationships that may exist between the indicator concentrations and the pathogens described in this study.

Third, the extent to which testing and subsequent removal of pathogen-positive boneless beef lots reduced the amount of pathogen contamination in ground beef is not clear. It is tempting to suggest that because of the differences in lot and sublot sizes tested (900 kg for boneless beef and 4,500 kg for ground beef), the reduction of *E. coli* O157:H7 and *Salmonella* between boneless and ground beef is greater than it first appears. However, differences in sampling confounds such comparisons. Work to mathematically model the impact of removing pathogen-positive boneless beef lots and pathogen-positive ground beef sublots on recipient exposure is needed.

Taken cumulatively, findings from this study suggest that beef produced for the NSLP and other federal nutrition assistance programs during school years 2015 through 2018 was done so under well-controlled food safety systems. Given the setting in which most beef procured by AMS is consumed—school cafeterias—any outbreak of illness is likely to be ascertained by public health authorities; yet to the best of our knowledge, no outbreaks of foodborne illness have been attributed to beef procured by AMS *(10)*. In an effort to ensure federal nutrition assistance program recipients receive safe food, AMS will continue to engage the full spectrum of stakeholders to refine and strengthen its beef purchase specifications, and it will work with partners at the Food and Nutrition Service and elsewhere to help provide safe food handling and related training to those who prepare and serve the beef it procures.

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