Journal of Food Protection, Vol. 82, No. 5, 2019, Pages 775–784
https://doi.org/10.4315/0362-028X.JFP-18-478

Research Note

Prevalence and Characterization of *Salmonella* Present during Veal Harvest

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MS 18-478: Received 3 October 2018/Accepted 31 December 2018/Published Online 15 April 2019

ABSTRACT

Beef and veal products have been vehicles implicated in the transmission of *Salmonella enterica,* a gastroenteritis-causing bacteria. Recent regulatory samples collected from veal have indicated bob veal, or calves harvested within days of birth, have higher rates of *Salmonella* than samples collected from formula-fed veal, or calves raised 20 weeks on milk replacer formula before harvest. To investigate this problem, we collected samples from veal calf hides, preevisceration carcasses, and final carcasses at five veal processors that harvested bob or formula-fed veal or both. Prevalence and concentrations of *Salmonella* were determined, and then the isolates were characterized for serovar and antibiotic susceptibility. *Salmonella* was more prevalent ($P < 0.05$) among bob veal than formula-fed veal hides, preevisceration carcasses, and final carcass (84.2 versus 15.6%, 62.8 versus 10.1%, and 12.0 versus 0.4%, respectively). Concentrations of *Salmonella* could be estimated by using regression order statistics on hides and preevisceration carcasses at two veal plants, with one harvesting bob veal and the other bob and formula-fed veal. The concentration of *Salmonella* on bob veal hides at the plants was 1.45 ± 0.70 and 2.04 ± 1.00 log CFU/100 cm^2, greater ($P < 0.05$) than on formula-fed veal hides, which was 1.10 ± 1.51 log CFU/100 cm^2. Concentrations on carcasses, however, were very low. Seventeen *Salmonella* serovars were identified among 710 isolates. *Salmonella* serovars London, Cerro, and Muenster were most common to bob veal and made up 50.7, 18.7, and 6.3% of the isolates, respectively, while serovar Montevideo (6.8% of isolates) was most common to formula-fed veal. Although bob veal had increased prevalence and concentrations of *Salmonella,* one group of formula-fed veal was found to harbor human disease–related antibiotic-resistant *Salmonella* serovars Heidelberg and the monophasic variant of Typhimurium (1,4,[5],12:i:–). Veal processors have made changes to improve the safety of veal, but further efforts are necessary from both bob and formula-fed veal to address *Salmonella*.

Key words: Processing; *Salmonella*; Veal

Salmonella enterica is estimated to cause 1.2 million cases of gastroenteritis, 23,000 hospitalizations, and 450 deaths each year in the United States *(39)*. Cattle are among the multitude of *Salmonella* reservoirs; thus, ground beef has been implicated as a mode of transmission in foodborne outbreaks *(13, 32)*. The implementation of hazard analysis and critical control point plans at large and small beef processors has decreased *Salmonella* in ground beef by 30% (7.5 to 5.8%) *(19)*. Since the Food Safety and Inspection Service (FSIS) published its approach to control the entry of *Salmonella* into the meat supply, the monitoring and regulation of *Salmonella* has become more stringent *(43),* yet the prevalence of antimicrobial-resistant strains is of constant concern.

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Surveillance data from the National Antimicrobial Resistance Monitoring System, which tracks antimicrobial resistance (AMR) in bacteria isolated from humans, animals, and foods, has shown a continued increase in the proportion of AMR in *Salmonella (14)*. This is a concern because AMR strains may cause more severe and prolonged disease than the antimicrobial susceptible strains *(42, 48)*. AMR in *Salmonella* is not only a public health concern but also a food safety concern, as a 2002 outbreak linked to AMR in *Salmonella enterica* serovar Newport in ground beef caused 47 illnesses, 17 hospitalizations, and one death *(12)*.

Veal is the meat of young cattle or calves, mostly of dairy cattle breeds, of which approximately 45,000 metric tons are consumed in the United States each year. Two types of veal are generally harvested in the United States, bob veal (calves sold and harvested within the first few days of

life), and formula-fed veal (calves raised on a milk replacer diet until harvested at about 20 weeks of age *(44)*). Since June 2012, FSIS has increased its regulatory attention on veal due to a higher percentage of non-O157 Shiga toxin–producing *Escherichia coli*–positive samples collected from veal trimmings than from products produced from other classes of cattle. This increased attention has revealed that although veal carcasses have a lower overall prevalence of *Salmonella* compared with beef carcasses *(47),* the portion that is bob veal relative to formula-fed veal is more contaminated with *Salmonella (29)*.

Although *Salmonella* has long been identified as a problem in veal *(30, 36, 41, 51),* its prevalence during the various steps of modern U.S. veal processing has not been examined, and the biological rationale for the higher positive rate in bob veal relative to formula-fed veal and beef is unclear. Therefore, the objectives of this study were to (i) assess the rates and concentrations of *Salmonella* present on veal calf hides, veal preevisceration carcasses, and final chilled veal carcasses and (ii) characterize the serovars and antibiotic susceptibility of any *Salmonella* identified. To accomplish this goal, samples collected at five veal processors, which harvested formula-fed veal or bob veal or both, were examined for prevalence and characterization of *Salmonella*.

MATERIALS AND METHODS

Design. Five veal processors that harvested formula-fed or bob veal or both were visited once (two of the processors) or twice (three of the processors), and samples were collected from hides, preevisceration carcasses, and final carcasses to determine the prevalence and concentration of *Salmonella* at each plant for each sample and veal type. *Salmonella* isolates were characterized for serovar and antibiotic sensitivity.

Sample collection and processing. Hide, preevisceration carcass, and final carcass samples were collected as previously described *(8)* by using buffered peptone water–moistened (BD, Sparks, MD) Speci-Sponges in Whirl-Pak bags (Nasco, Fort Atkinson, WI). Hide samples were obtained by swabbing an area (600 cm^2) over the brisket-plate region after stunning and exsanguination. Preevisceration carcass samples were collected as soon as possible after complete removal of the hide and final carcass samples were collected from chilled or chilling carcasses as they entered the cooler. The carcass samples were collected from the brisket-plate and hock-round areas of one side of each carcass from an area of 6,000 $\pm$ 100 cm^2 for formula-fed veal carcasses and 2,000 $\pm$ 200 cm^2 for bob veal carcasses. Carcass samples were not directly matched to one another or to hide samples, but all samples were collected during one shift at each processor. All sponge samples were shipped to the laboratory in insulated coolers via overnight courier. On receipt in the laboratory, each sample was hand massaged thoroughly in the bag, and an aliquot (1.0 mL from hide samples and 4.0 mL from preevisceration and final carcasses samples) removed for enumeration of *Salmonella* as described in the following. After the aliquots for enumeration were removed from each sample, 80 mL of tryptic soy broth (TSB; BD) was added to each sample for nonselective enrichment to determine the prevalence of *Salmonella* as described in the following.

Enumeration of *Salmonella*. To enumerate the amount of *Salmonella* on hides, a 50-μL portion of each sample was spiral plated onto one xylose lysine desoxycholate medium (Oxoid, Remel, Lenexa, KS) plate containing 4.6 mL/L Tergitol, 15 mg/L novobiocin, and 5 mg/L cefsulodin (XLD_{tnc}; MilliporeSigma, St. Louis, MO) *(9)*. Plates were incubated at 37°C for 18 to 22 h, and presumptive colonies were counted and confirmed as *Salmonella* by PCR amplification of the *Salmonella*-specific *invA* target *(50)*. To enumerate the amount of *Salmonella* on preevisceration carcasses, a 1-mL portion of each sample was dispensed onto Petrifilm EB (3M Microbiology, St. Paul, MN) for the growth of *Enterobacteriaceae*. The Petrifilm was incubated at 37°C for 15 to 20 h and then held at 4°C (approximately 48 h) until the results of *Salmonella* isolation were complete. Then, all carcass samples found culture positive for *Salmonella* had their corresponding Petrifilm plates replica plated onto XLD_{tnc}, as described previously *(10)*. The XLD_{tnc} plates were incubated, and suspect colonies counted and confirmed as described previously. When present, up to 10 colonies were selected for confirmation. If any suspect colonies did not confirm to be *Salmonella,* the concentration was calculated by multiplying the number of suspect colonies by the number confirmed divided by the number tested.

Determination of *Salmonella* prevalence. After 80 mL of TSB (see earlier text) was added, each sponge sample was enriched as previously described *(8)*. Then, a 1-mL aliquot of the TSB enrichment was removed from each sample for immunomagnetic concentration, selective enrichment in Rappaport-Vassiliadis soya broth (Oxoid), and streaked for isolation onto an XLD_{tnc} plate as previously described *(11)*. Plates were viewed for presumptive *Salmonella* (black colonies), and then two well-isolated presumptive colonies per plate were arbitrarily selected and picked with a sterile loop to a 96-well block of TSB (0.5 mL per well). The 96-well TSB blocks were incubated at 37°C overnight (15 to 22 h), and then 2 μL was removed from each well and the isolate confirmed to be *Salmonella* by using PCR as described previously *(50)*.

Serotyping of *Salmonella* isolates. One confirmed *Salmonella* isolate per positive enumeration or prevalence sample was streaked for isolation onto tryptic soy agar (TSA; BD) and incubated at 37°C for 18 to 20 h, and then the serovar was determined by using the molecular methods as previously described *(20, 26, 27)*. Resulting serovars were confirmed by using slide agglutination (O typing) and tube agglutination (flagellar H-typing) methods, with commercial antisera (Difco, BD Diagnostic Systems, Sparks, MD), following manufacturer's guidelines. When molecular methods were not confirmed or when molecular methods did not provide a serovar for an isolate, Wellcolex Color *Salmonella* agglutination (Remel) and traditional slide agglutination O grouping and tube agglutination flagellar H typing were performed and considered as the correct serovar.

Susceptibility screening. Each isolate of *Salmonella* was inoculated into a well of a 96-well block containing TSB. This block was incubated overnight at 37°C, and then 10 μL from each well was transferred to a second 96-well block containing 1 mL of buffered peptone water in each well. The diluted (1:100) *Salmonella* strains were screened for resistance to antibiotics by using a 96-pin Boekel microplate replicator (Boekel Scientific, Feasterville, PA) to inoculate three 150-mm TSA plates supplemented with either 32 mg/L tetracycline (Tet), 32 mg/L nalidixic acid (Nal), or 2 mg/L cefotaxime (Ctx), and one nonsupplemented TSA plate that served as a growth control. Inoculated plates were

TABLE 1. *Prevalence of* Salmonella *on bob and formula-fed veal hides and carcasses at five processors over two sample collections*

				% *Salmonella* prevalence[c]		
					Carcass:	
Plant	Sampling[a]	Type[b]	*n*	Hide	Preevisceration	Final
A	Initial	Formula-fed	90	3.3 D[d]	0.0 D	0.0 C
B	Initial	Bob	95	100.0 A	70.5 B	7.4 B
B	Follow-up	Bob	95	52.6 B	21.1 C	7.4 B
C	Initial	Formula-fed	95	0.0 D	0.0 D	0.0 C
C	Follow-up	Formula-fed	95	26.3 C	3.2 D	0.0 C
D	Initial	Formula-fed	95	1.1 D	0.0 D	0.0 C
D	Follow-up	Formula-fed	95	4.2 D	1.1 D	0.0 C
E	Initial	Bob	95	100.0 A	96.8 A	21.1 A
E	Initial	Formula-fed	48	100.0 A	100.0 A	4.2 B C
A			90	3.3 O	0.0 N	0.0 N
B			190	76.3 M	45.8 M	7.4 M
C			190	13.2 N	1.6 N	0.0 N
D			190	2.7 O	0.6 N	0.0 N
E			143	100.0 L	97.9 L	15.4 L
	Initial		285	33.7 Q	23.5 Q	2.5 Q
	Follow-up		285	27.7 Q	8.5 R	2.5 Q
		Formula-fed	518	15.6 Y	10.1 Y	0.4 Y
		Bob	285	84.2 X	62.8 X	12.0 X
All combined			803	39.9	28.5	4.5

[a] There were two sample collections performed at three of the plants approximately 12 months later.
[b] Type of veal: bob, calves younger than 2 weeks old; formula-fed, calves raised on milk replacer formula for 20 to 22 weeks.
[c] Values represent the percentage of samples found to be positive for *Salmonella* by culture isolation. Preevisceration, carcass after hide was fully removed and before interventions were applied.
[d] Within each section and within a column, values followed by the same letter (A through D, L though O, Q and R, and X and Y) are not different ($P > 0.05$).

incubated at 37°C for 16 h and then viewed for growth. Isolates were considered resistant to the selected antibiotic if growth on antibiotic-containing agar was visually the same as growth on control agar lacking antibiotics. If small microcolonies were present, the growth was interpreted as sensitive to the antibiotic.

Statistical analysis. Analysis of variance of *Salmonella* prevalence and mean concentrations of *Salmonella* (log transformed) for each sample type by plant and veal type (formula-fed and bob) was performed by using GraphPad Prism Software (GraphPad Software, La Jolla, CA). Specifically, the nonparametric data were analyzed by using Kruskal-Wallis one-way analysis of variance by ranks with Dunn's multiple comparison posttest, with $P < 0.05$ being significant. For the concentration of *Salmonella,* all culture-positive samples that were nonenumerable were assigned an arbitrary value of one-half the level of detection (LOD). Then, the "regression on order statistics" (ROS) method for censored analysis was used to calculate summary statistics (e.g., log mean and standard deviation). This approach uses the information that is available in nonenumerable but positive data by assuming microbial concentrations fit a parametric distribution (log normal). Specifically, a linear regression is used to fit the normal quantiles of the censored and noncensored log data to obtain a more accurate mean and standard deviation than substitution or omission *(25)*.

RESULTS AND DISCUSSION

Prevalence of *Salmonella* contamination. FSIS has maintained increased attention to veal processing and products since 2012 when veal samples were observed to have a higher percentage of non-O157 Shiga toxin–producing *E. coli* than beef samples *(44, 45)*. This scrutiny later lead to observations of *Salmonella* being disproportionately higher in bob veal compared with other forms of veal *(29, 46)*. Therefore, during other studies of veal processing to assess efficacy of sanitary dressing procedures at five veal processors *(8),* we examined the prevalence, concentration, and types of *Salmonella* on hides, preevisceration carcasses, and final carcasses.

As hide is the general source of *E. coli* and *Salmonella* contamination in beef processing *(5, 7, 37),* we examined veal hides. Overall, 39.9% of veal hides were positive for *Salmonella* (Table 1). This varied significantly by plant, sample collection trip, and veal type. Plants that harvested bob veal had higher hide prevalence (145 of 190, 76.3%, and 143 of 143, 100%) than those that exclusively harvested formula-fed veal (5 of 190, 2.7%; 3 of 90, 3.3%, and 25 of 190, 13.2%). However, follow-up hide samples showed both veal types could present with a different ($P < 0.05$)

prevalence of *Salmonella* on their hides at any given time. All the initial bob veal hide samples collected at plant B were positive (95 of 95, 100%), while 50 (52.6%) of 95 follow-up samples were positive. Conversely, no *Salmonella* was found on formula-fed veal hides during the initial collection at plant C (0 of 95), but 25 (26.3%) of 95 of hides were *Salmonella* positive during the follow-up sample collection. Overall, the hide prevalence of *Salmonella* between the initial and follow-up samples at all plants combined was lower but not significantly different ($P > 0.05$). Although the difference in hide *Salmonella* between all bob and formula-fed veal was significant ($P < 0.05$), there was no significant difference between veal types at plant E ($P > 0.05$), where both types of veal were harvested. At plant E, all hides of bob (95 of 95) and formula-fed (48 of 48) veal were *Salmonella* positive, possibly as a result of cross-contamination in lairage environments *(1)*.

The prevalence of *Salmonella* on preevisceration carcasses followed what was observed for hides. The highest prevalence of *Salmonella* on preevisceration carcasses was at plants B and E, which harvested bob veal and where 87 (45.8%) of 190 and 140 (97.9%) of 143 of preevisceration carcasses were *Salmonella* positive (Table 1). Although the initial and follow-up sample collections showed no difference in hide *Salmonella* prevalence, there was a significant difference ($P < 0.05$) in preevisceration carcass prevalence at 67 (23.5%) of 285 versus 24 (8.5%) of 285. Our follow-up samples were collected, in part, to examine changes made at plants B, C, and D in sanitary dressing procedures following the initial sample collection *(8)*. Indeed, the rates of hide-to-carcass transfer (HTCT) as calculated by the ratio of preevisceration carcass prevalence to hide prevalence of *Salmonella (11)* was 0.697 initially and 0.307 after changes to dressing procedures were made (data not shown). This reduction in HTCT was primarily impacted by changes in the contamination of bob veal carcasses at plant B.

The prevalence of *Salmonella* on finished veal carcasses was only observed at plants B and E, where bob veal was harvested and where preevisceration carcass contamination was increased (Table 1). Overall, 34 (12.0%) of 285 bob veal final carcasses were *Salmonella* positive, while only 2 (0.4%) of 518 formula-fed veal final carcasses were. Despite improvements to dressing procedures that resulted in lower preevisceration carcass *Salmonella* at plant B, the final carcasses were unchanged in *Salmonella* prevalence between the two sample collections. The reason for this is not clear. We suspect that postevisceration processing interventions were less effective or inconsistently delivered when applied to smaller bob veal carcasses. This may be why at plant E, where nearly all preevisceration carcasses of both bob and formula-fed veal were contaminated with *Salmonella,* about three times as many bob veal final carcasses were *Salmonella* positive after traveling the same processing line as compared with formula-fed veal carcasses. Another likely cause could be variability in the slaughter facilities. For example, effectiveness may have increased overall, but sampling during the follow-up happened during an unusually high contamination period or during poor sanitation conditions that day. Incoming bob veal that had a higher estimated concentration of *Salmonella* present but with much more variability is discussed later. Because there was so little *Salmonella* detected on final carcasses, variability in sample collections is just as likely as other hypothetical causes.

Salmonella was found at four of the five veal plants visited; however, its prevalence at two of the plants (A and D) was negligible compared with the 100% prevalence observed on hides at plants B and E. Again, this observation supports the findings of FSIS in that the increased prevalence of *Salmonella* is associated with bob veal calves. The prevalence of *Salmonella* on the hides of cull cattle and cull cows has been reported to similarly range from 70 to 100% *(4, 11)*. The HTCT of *Salmonella* was significant with the preevisceration carcass prevalence of *Salmonella* at 45.8 and 97.9% for plants B and E, respectively. When hide *Salmonella* prevalence of 100% was previously reported for small processing plants, preevisceration carcass prevalence of *Salmonella* was observed to vary by plant from 93.7% to 80.4 and 72.6% *(4)*. At plants A and D, where hide *Salmonella* prevalence was 3.3 and 2.7%, only one preevisceration carcass was found to be contaminated by *Salmonella*.

Concentrations of *Salmonella* contamination. In addition to measuring the prevalence of *Salmonella* contaminating veal at the five processors, we also determined the load of *Salmonella* present through enumeration (Table 2). Measurable concentrations of *Salmonella* were only found on hides and preevisceration carcasses at plants B and E, corresponding to the plants that harvested bob veal. Using ROS *(25),* the mean concentration of *Salmonella* on hides was estimated to be 1.10 to 2.04 log CFU/100 cm^2, while the mean concentration on preevisceration carcasses was highly variable, with estimates ranging from –5.25 to –1.51 log CFU/100 cm^2.

We used ROS to estimate the concentrations of *Salmonella* on the hides and carcasses because values determined by ROS are more accurate in situations with results such as ours *(25)*. Generally, when calculating mean concentrations, there is a problem when considering samples that are culture positive but nonenumerable, that is, at a concentration less than the LOD of the enumeration method. Our culture method has been validated to detect 1 to 3 CFU in a sample; thus, the LOD of culture was orders of magnitude lower than the enumeration method. For example, a hide sample could have been culture positive if 1 CFU of *Salmonella* were present, whereas approximately 20,000 CFU were required present to reach the LOD of the enumeration method. A common practice to address this situation in data analysis is to replace the unknown concentrations with a value that is half (50%) of the LOD; however, that results in an artificially high estimate of the concentration and masks the variability present in the data *(25)*. By using ROS, instead of Kaplan-Meier statistics, we could examine events when there were less than 50% enumerated values, as ROS is more robust and provides

TABLE 2. *Concentrations of* Salmonella *on bob and formula-fed veal hides and preevisceration carcasses at five processors over two sample collections*[a]

Plant	Sample[b]	Type[c]	*n*	No. positive	No. enumerable	Mean[d]	SD[d]	Range[e]
Hide								
A	Initial	Formula-fed	90	3	0			−0.78 to 1.82
B	Initial	Bob	95	95	39	1.45	0.70	−0.78 to 3.60
B	Follow-up	Bob	95	49	1			−0.78 to 1.82
C	Initial	Formula-fed	95	0				
C	Follow-up	Formula-fed	95	25	0			−0.78 to 1.82
D	Initial	Formula-fed	95	1	0			−0.78 to 1.82
D	Follow-up	Formula-fed	95	4	0			−0.78 to 1.82
E	Initial	Bob	95	95	60	2.04	1.00	−0.78 to 5.39
E	Initial	Formula-fed	48	48	8	1.10	1.51	−0.78 to 4.00
Preevisceration carcass								
A	Initial	Formula-fed	90	0				
B	Initial	Bob	95	66	4	−5.25	2.74	−0.88 to 1.62
B	Follow-up	Bob	95	20	3	−1.51	0.72	−0.88 to 0.30
C	Initial	Formula-fed	95	0				
C	Follow-up	Formula-fed	95	3	0			−1.48 to −0.48
D	Initial	Formula-fed	95	0				
D	Follow-up	Formula-fed	95	1	0			−1.48 to −0.48
E	Initial	Bob	95	91	7	−4.75	2.81	−0.88 to 2.15
E	Initial	Formula-fed	48	48	3	−2.04	0.92	−1.48 to 0.00

[a] Only *Salmonella* concentrations from hides and preevisceration carcasses presented as final carcasses lacked any enumerable amounts of *Salmonella* to allow calculation.
[b] There were two sample collections performed at three of the plants approximately 12 months later.
[c] Type of veal: bob, calves younger than 2 weeks old; formula-fed, calves raised on milk replacer formula for 20 to 22 weeks.
[d] Means and standard deviations (SD) calculated by ROS method of censored data.
[e] Range provides the concentrations of *Salmonella* used for calculations. Low value represents 1 CFU of *Salmonella* in sample to provide culture positive; high value represents the enumeration level of detection (LOD) or greatest measured concentration. The LOD for hides was 1.82 log CFU/100 cm^2, LOD of bob veal carcasses was 0.00 log CFU/100 cm^2, and LOD of formula-fed veal carcasses was –0.48 log CFU/100 cm^2.

good estimates down to about 20% enumerated values *(25)*. Our preevisceration carcass enumeration results, however, were still out of the recommend range, even for ROS, so calculated mean concentrations should not be taken directly, but rather qualitatively. As a whole, the ROS estimated concentrations of *Salmonella* on preevisceration carcasses support the premise that the sanitary dressing practices and antimicrobial interventions were preventing and reducing HTCT, but there remains intermittent clusters of contamination.

When examining the number of hides that were enumerable, 28% (39 + 1 of 95 + 49 [40 of 144]) and 63% (60 of 95) bob veal hides at plants B and E, respectively (Table 2), had *Salmonella* present at a concentration greater than our limit of detection (LOD = 1.8 log CFU/100 cm^2). At plant E, the difference between bob veal hides and formula-fed veal hides was significantly different ($P < 0.05$) for the number that were enumerable, as well as the concentration of *Salmonella* present. Significant differences ($P < 0.05$) in the number of enumerable hides at plant B were also seen between the two collection times at that plant. Thirty-nine (41%) of 95 bob veal hides were enumerable at plant B during the initial sample collection, while only 1 (2%) of 49 of the culture-positive bob veal hides on the second sample collection were enumerable. This veal processor had made changes to their processes between the two sample collections that included optimizing a hide-directed intervention *(49),* and this may have contributed to the decreased hide *Salmonella* concentration measured on the follow-up sampling.

Only 14 (8%) of 177 bob veal preevisceration carcasses had enumerable *Salmonella* (Table 2); however, the difference between the two sample collections at plant B was significantly different ($P < 0.05$) in this regard with 4 (6%) of 66 initially and 3 (15%) of 20 on follow-up. The ROS mean concentration on the carcasses was different as well between the two sampling periods, and although the number of *Salmonella* prevalence positive carcasses was reduced in the second sample collection, the proportion that was enumerable was greater. Due to differences in carcass sizes, the surface area sampled was different between bob and formula-fed veal carcasses such that the LOD of *Salmonella* on bob veal carcasses was 0.0 log CFU/100 cm^2, while the LOD for formula-fed veal carcasses was –0.48 log CFU/100 cm^2. If it had not been for the increased sample area and lower LOD, enumerable formula-fed veal carcasses would likely not have been identified. No final veal carcasses had enumerable amounts of *Salmonella* present.

In comparison to other studies using similar enumeration methods, 9.4 to 21.3% of cull cattle hides were found to be enumerable for *Salmonella* during different seasons of the year, and the observed mean load was 1.9 to 2.2 log CFU/100 cm^2, with individual measurements ranging from 1.6 to 4.5 log CFU/100 cm^2 *(11)*. In a study of seven small processing plants, 36.6% of hides were found enumerable for *Salmonella,* with a range of 1.6 to 5.6 log CFU/100 cm^2 *(4)*. For preevisceration cull cattle carcasses, 5.1 and 13.0% were found enumerable at concentrations ranging from –0.3 to 2.7 log CFU/100 cm^2 *(11)*. Fourteen percent of *Salmonella*-positive preevisceration carcasses at seven small processors were only enumerable at concentrations ranging from –0.3 to 2.9 log CFU/100 cm^2 *(4)*. These previous studies determined mean concentrations of *Salmonella* by using only the enumerable sample values or censored data, whereas ROS was used here to provide a more accurate estimate of *Salmonella* concentrations on hides and carcasses. With the exception of the rates of enumerable *Salmonella* on bob veal hides being greater, the rate and the concentrations of enumerable *Salmonella* on preevisceration beef carcasses are similar to those of bob veal. Information on *Salmonella* prevalence and concentrations on finished veal carcasses is limited. In their beef and veal baseline survey report *(46),* FSIS found 15% of *Salmonella*-positive prechill veal carcasses enumerable at a mean concentration of 0.28 log CFU/100 cm^2, while 12% of prechill beef carcasses were enumerable at a mean concentration of 0.19 log CFU/100 cm^2. These concentrations were determined by using a most-probable-number (MPN) method *(46),* and direct comparisons to our values should be made with caution as injured cells can more easily recover and grow for detection in the MPN method compared with the direct plating assay *(33)*.

These results also are consistent with those of Arthur et al. *(2),* indicating lower hide concentrations of a pathogen reduces the rate of HTCT. Veal processing is more challenging than beef processing due to the differences in the hides that must be removed during processing *(49)*. Hide removal is the primary contributor to carcass contamination in beef processing *(5, 7, 37)*. Thus, with similar concentrations on hides, it is likely that the hide removal process from bob veal is responsible for contamination of bob veal carcasses, as illustrated in the data from plant E. Further, bob veal carcasses are much smaller than formula-fed veal carcasses. During our study, when bob veal carcasses were observed through processing, the carcasses shifted and changed position by swinging and swaying during hide removal and while encountering spray interventions. This may have caused more HTCT and hindered the efficacy of the carcass-directed interventions.

FSIS has reported in their beef and veal baseline survey report *(46)* that veal carcasses have a lower prevalence of *Salmonella* than beef carcasses and that the majority of *Salmonella*-contaminated veal carcasses are bob veal as opposed to formula-fed veal *(29)*. Bob veal calves are only days old at harvest, and these young nonruminating calves harvested for veal are more easily colonized by *Salmonella*. Young animals are frequently colonized and are most likely to experience salmonellosis within 2 to 4 weeks of age *(28),* evidenced by *Salmonella* outbreaks in young calves and calf ranches that can lead to high amounts of *Salmonella* present during veal processing *(34)*. Colonization can occur through exposure to asymptomatic carriers, such as adult cattle infected by *Salmonella* but showing no clinical signs of infection *(23)*. Alternately, evidence has been reported that neonatal calves can acquire *Salmonella* through vertical transmission and already be infected at birth *(23, 24)*. The lower rates of *Salmonella* in formula-fed veal is likely related to their rearing in barns and individual or low-density pens. *Salmonella* on the hide of a calf may not survive for long periods similar to *E. coli* O157:H7 *(3)* such that by 20 to 22 weeks of age, any *Salmonella* present no longer persists among the animals.

Characterization of *Salmonella* serovars. From each positive enumeration and prevalence sample, one *Salmonella* isolate was selected to identify its serovar (Table 3) and screened for resistance to Ctx, Nal, and Tet. This resulted in 710 total isolates, with 125 from enumeration and 585 from prevalence. Because of the greater prevalence and concentrations of *Salmonella* in bob veal relative to formula-fed veal, there were 561 bob veal isolates and 149 formula-fed veal isolates. In addition, plant E accounted for 109 of the formula-fed veal isolates. As mentioned earlier, many of these formula-fed veal isolates at plant E may arguably be considered the result of cross-contamination from bob veal calves in the lairage pens, as the bob veal were processed before the formula-fed veal.

Seventeen different *Salmonella* serovars were identified (Table 3), with the most common serovar identified as *Salmonella* London, which accounted for 360 (50.7%) of 710 of all the *Salmonella* isolates. This serovar was only found at plants B and E and has not been reported to be commonly isolated from beef or cattle in the United States *(4, 6, 9, 11)*. Human infections by *Salmonella* London have been infrequently reported by the Centers for Disease Control and Prevention *(15)*; however, it was associated with a Korean outbreak in 2000 traced to infant formula *(31, 38)*. The next most common serovars were *Salmonella* Cerro (133 of 710, 18.7%), Montevideo (48 of 710, 6.8%), Muenster (45 of 710, 6.3%), and Agona (30 of 710, 4.2%), which collectively accounted for an additional 36% of the isolates. These four serovars are commonly identified in beef products and cattle *(4, 6, 9, 11),* and *Salmonella* Montevideo and Agona are among the 20 most prevalent serovars reported by the Centers for Disease Control and Prevention *(15)*. In most cases, the serovar of *Salmonella* isolated from a final carcass at a plant was also identified on preevisceration carcasses at that plant, and, likewise, a serovar of *Salmonella* isolated from a preevisceration carcass was also identified on hides at that plant.

When a serovar was observed more than once, it was seen at plants B and E (Table 3), where most of the isolates were found in the first place. The *Salmonella* serovars identified in the follow-up samples at plants B, C, and D were often different from those found during the initial sample collection, showing that the *Salmonella* was highly

TABLE 3. Salmonella *serovar distribution of isolates from enumeration and prevalence positive samples of hides, preevisceration carcasses, and final carcasses collected from bob and formula-fed veal calves at five processors during two sample collections*[a]

	Plant and collection trip observed[b]								Bob veal[c]				Formula-fed veal[d]			
Salmonella serovar	A1	B1	B2	C1	C2	D1	D2	E1	*n*	Hide	Pre	Final	*n*	Hide	Pre	Final
Agona ($n = 30$)	+	+						+	16	7	9	0	14	9	5	0
Altona ($n = 2$)								+	2	0	2	0	0	0	0	0
Amager ($n = 1$)			+						1	1	0	0	0	0	0	0
Anatum ($n = 13$)								+	10	8	2	0	3	3	0	0
Cerro ($n = 133$)		+	+					+	132	84	34	14	1	0	1	0
Dublin ($n = 21$)			+						1	1	0	0	0	0	0	0
Give ($n = 3$)		+						+	3	1	0	2	0	0	0	0
Heidelberg ($n = 15$)					+				0	0	0	0	15	12	3	0
London ($n = 360$)		+						+	316	177	128	11	44	19	24	1
Mbandaka ($n = 7$)		+						+	7	4	3	0	0	0	0	0
Meleagridis ($n = 2$)		+							2	2	0	0	0	0	0	0
Montevidio ($n = 48$)		+	+					+	8	5	2	1	40	23	16	1
Muenster ($n = 45$)		+						+	45	36	5	4	0	0	0	0
Newport ($n = 12$)			+					+	4	1	3	0	8	3	5	0
Stanleyville ($n = 1$)			+						1	1	0	0	0	0	0	0
Typhimurium ($n = 20$)		+	+				+		15	11	2	2	5	4	1	0
Typhimurium 1,2 null ($n = 14$)[e]					+				0	0	0	0	14	14	0	0
ND ($n = 5$)[f]								+	0	0	0	0	5	5	0	0
Total ($n = 710$)	+	+	+	+	+	+	+	+	561	339	190	34	149	92	55	2

[a] Values represent the number of isolates of each serovar identified. Pre, preevisceration carcasses; Final, final carcasses.
[b] Five veal processing plants (A through E) were visited for initial samples (1). There was a second sample collection (2) performed at three of the plants approximately 12 months later. +, collection trip serovar was identified.
[c] Bob veal are calves younger than 2 weeks old at harvest.
[d] Formula-fed veal are calves raised on milk replacer formula for 20 to 22 weeks before harvest.
[e] Monophasic variant of *Salmonella enterica* serovar Typhimurium (1,4,[5],12:i:–).
[f] ND, *Salmonella* serovar could not be determined.

variable and likely endemic to the various incoming sources of veal calves. *Salmonella* Agona was the only serovar observed at three different plants (Table 3). *Salmonella* Heidelberg and a monophasic variant of serovar Typhimurium (1,4,[5],12:i:–), hereafter referred to as Typhimurium 1,2 null, were only found during follow-up sample collection at the formula-fed veal plant C. When the two sample collections are compared, *Salmonella* serovars Cerro, Montevideo, and Typhimurium were observed during both collections at bob veal plant B. *Salmonella* serovars Cerro and Typhimurium were present at enumerable concentrations on hides and carcasses at plant B and only on bob veal hides and carcasses at plant E, while they were not present at these concentrations on the formula-fed veal at plant E. Although *Salmonella* serovar Montevideo was present on 23 of the 48 formula-fed veal hide samples at plant E, and at enumerable levels in two of those cases, it was only observed on bob veal hides at plant B five times over the two sample collections (Table 3). These observations suggest that particular serovars of *Salmonella* may be attributed to certain types of veal; however, given the limited number of samples in our study, drawing such conclusions should be weighed with caution until further research has been performed.

Susceptibility of *Salmonella* isolates. All the *Salmonella* isolates were screened for resistance to Ctx, Nal, and Tet (data not shown). In total, 91 isolates representing 13 serovars demonstrated resistance by growing on one or more agar containing each of the antibiotics. Only *Salmonella* serovars Altona, Anatum, Give, and Stanleyville were absent in the resistant group. A significantly greater proportion ($P < 0.05$) of isolates from formula-fed veal (40 of 149, 26.8%) compared with bob veal (54 of 561, 9.6%) grew in the presence of one or more of the antibiotics. Isolates were screened against Tet as an indicator of general AMR, as it is one of the most commonly observed resistances *(40)*. Thirty-seven isolates (33 from bob veal and 4 from formula-fed veal) were only resistant to Tet, while the remainder were resistant to Ctx or Nal or both. The group of isolates only resistant to Tet were of seven different serovars. Similarly, in a study of grain-fed veal calves, resistance to Tet was observed to be the most common resistance alone and in combination with other antimicrobials among four reported serovars *(18)*.

We screened for resistance to Ctx and Nal as indicators of resistance to significant human-use antibiotics *(21, 40)*. This method of screening is rapid but crude. Further investigation of these isolates appearing resistant to Ctx and Nal through the use of MIC panels of antibiotics or molecular screening for specific genes related to the resistance is required before they can be definitively categorized. Therefore, isolates that demonstrated growth on Ctx plates or Nal plates or both can only be considered

putatively resistant. Thirty-five isolates grew on Ctx plates, 18 grew on Nal plates, and one isolate grew on both. *Salmonella* serovars with isolates putatively resistant to Ctx were Agona ($n = 12$), Typhimurium 1,2 null ($n = 13$), Typhimurium ($n = 7$), and Cerro ($n = 2$). *Salmonella* serovars with isolates putatively resistant to Nal were Heidelberg ($n = 13$), London ($n = 2$), Typhimurium ($n = 2$), and Cerro ($n = 1$). The isolate putatively resistant to both Ctx and Nal was *Salmonella* serovar Typhimurium 1,2 null. The occurrence of these resistant serovars was specific to plant and veal calf production group. All of the identified *Salmonella* Heidelberg isolates were from formula-fed veal plants C ($n = 13$) and D ($n = 1$). All of the identified *Salmonella* Typhimurium 1,2 null isolates were found at plant C as well. Overall, the isolates that screened putatively resistant to significant human-use antibiotics were a greater proportion of formula-fed veal isolates (22.1%) compared with bob veal isolates (21 of 561, 3.7%).

The isolates of *Salmonella* serovars Heidelberg and Typhimurium 1,2 null are of particular interest from a public health stand point. *Salmonella* Heidelberg infected a total of 56 people from 15 states during an outbreak in 2015 to 2017 linked to contact with dairy calves *(16)*. Thirty-five percent of people in this outbreak were hospitalized, and no deaths were reported. The *Salmonella* Heidelberg isolates in this outbreak were resistant to multiple antibiotics, including Tet and Nal *(16)*. Serovar Typhimurium 1,2 null is a monophasic strain of *Salmonella* and has been reported to be of increasing concern in the European Union. A similar monophasic *Salmonella* serovar Typhimurium 1,2 null caused two major outbreaks, involving 133 confirmed cases, 24 hospitalizations, and one death in Luxembourg in 2006 *(35)*. In Italy, the *Salmonella* serovar Typhimurium 1,2 null represented the third most frequent serovar isolated from human cases between 2004 and 2008 *(17)*. Unlike the isolates in our study that were putatively resistant to Tet and Cef but sensitive to Nal, clinical isolates of Typhimurium 1,2 null that caused 206 illnesses in a 2013 to 2014 outbreak in central Italy were only resistant to Nal (*17*). In Denmark, serovar Typhimurium 1,2 null is reported to account for 20.5% of human *Salmonella* infections *(22)*. Most cases were often associated with consumption of pork and poultry rather than beef or veal. The *Salmonella* serovar Typhimurium 1,2 null in Denmark often exhibited antibiotic resistance and is considered an important epidemic health risk *(22)*.

The samples used in this study were originally collected to examine bacterial indicators of sanitary dressing and the efficacy of changes made to those practices *(8)* and were then later revisited to characterize *Salmonella* present on bob and formula-fed veal calf hides and its transmission to carcasses during the steps of harvest. As a result, the samples are limited in number and represent only five veal processing plants during one season of the year. Further, the numbers of bob veal to formula-fed veal are unbalanced and may not reflect the actual numbers of veal harvested. Note that the limited number of samples in our study only provide preliminary data, and larger systematic surveys of *Salmonella* in veal processing are needed to more fully understand this problem.

In conclusion, we visited five veal processors to assess prevalence and concentrations of *Salmonella* present at different stages of harvest of bob and formula-fed veal. Bob veal calves were found to have greater prevalence and concentrations of *Salmonella* on hides and carcasses than formula-fed veal. It is likely that the concentrations of *Salmonella* on hides and the challenges of hide removal from bob veal carcasses are combining to result in the increased prevalence FSIS has observed in veal. Although having less *Salmonella,* the formula-fed veal in our study was found to be contaminated by *Salmonella* serovars of a greater public health concern than those associated with bob veal. Some processors have already made changes that should improve the safety of veal, but further efforts are necessary from both bob and formula-fed veal processors.

ACKNOWLEDGMENTS

The authors thank the following: the cooperating veal processing plants for access to sample collection; the American Veal Association and its members for their contributions to this project; Greg Smith, Lawnie Luedtke, Bruce Jasch, and Frank Reno for technical support; and Jody Gallagher for secretarial assistance. Trade names are necessary to accurately report experimental results. The use of trade names by a U.S. Department of Agriculture (USDA) author implies no approval of the product to the exclusion of others that may also be suitable; further, the USDA neither guarantees nor warrants the standard of any products mentioned.

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