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Prevalence of *Salmonella* spp in cull (market) dairy cows at slaughter

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Objective—To determine the prevalence of *Salmonella* spp in the cecal-colon contents of cull (market) dairy cows at slaughter because of potential public health ramifications.

Design—Survey study.

Sample Population—Cecal-colon contents collected from 5,087 cull (market) dairy cows at slaughter at 5 slaughter establishments across the United States.

Procedure—During 2 periods of the year, winter (January and February) and summer (July through September), 5 cull (market) cow slaughter establishments in the United States—west (WE), southeast (SEE), central (CE), north central (NCE), and south central (SCE)—establishments were visited, and cecal-colon contents of cull dairy cows were obtained at the time of slaughter. Samples were examined by microbiologic culture at a single laboratory for *Salmonella* spp.

Results—*Salmonella* spp were detected in 23.1% of cecal-colon content samples from cull dairy cows across the 5 slaughter establishments. The highest site prevalence (54.5%) was detected at the WE during the summer period, whereas the lowest was found at the CE during the summer (4.3%) and at the NCE during the winter (4.5%). Considerable variation in the daily prevalence of *Salmonella* spp was found, particularly at the WE and the SCE. *Salmonella* spp were isolated from 93% of cecal-colon contents collected on a summer day at the WE.

Conclusions and Clinical Relevance—Results strongly suggest that there is a high prevalence of *Salmonella* spp in cull dairy cows at slaughter, which could burden Hazard Analysis Critical Control Point programs implemented in slaughter establishments. Procedures to reduce *Salmonella* load at the dairy farm and during transport to slaughter could reduce the risk of spread during the slaughter process. (*J Am Vet Med Assoc* 2001;219:1212–1215)

Salmonella spp infections within dairy operations may be widespread. Smith et al[1] found that 75% of dairies sampled in California had serologic evidence of recent exposure to *Salmonella* spp. *Salmonella* infections in dairy cows can pose substantial animal and public health threats.[1] For the period 1971 through 1983, cattle or their products (meat or milk) were incriminated as the source of infection in 14 of 38 (36.8%) outbreaks of human salmonellosis investigated by the **Centers for Disease Control and Prevention (CDC)**.[2] Cull (market) dairy cows account for large amounts of beef produced annually in the United States.[3] It has been estimated that approximately 17% of the nation's ground beef may come from cull dairy cows.[4] The consumption of undercooked ground beef is considered an important cause of salmonellosis in humans.[5,6] In this article, the designation "cull" dairy cow is used interchangeably with and means "market"[7] dairy cow.

It seemed appropriate to examine the prevalence of *Salmonella* spp in market dairy cows, because the national dairy herd includes approximately 9 million cows.[8] Culling rates seem to be high,[9] and there is concern that meat from culled dairy cows, especially ground beef, may be a source of *Salmonella* infection for humans. The purpose of the study reported here was to estimate the prevalence of *Salmonella* spp in market dairy cattle at slaughter at 5 nonfed beef slaughter establishments in the United States.

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Materials and Methods

Animals—A total of 5,087 cull dairy cows were sampled at 5 nonfed beef slaughter establishments representing 5 regions of the United States: **west establishment (WE)**, **southeast establishment (SEE)**, **central establishment (CE)**, **north central establishment (NCE)**, and **south central establishment (SCE)**. Samples were obtained from cows at each establishment twice in 1996, once during January or February (winter) and again during July through September (summer). Investigators collected samples at each establishment for 3 to 5 days during each period until approximately 500 cull dairy cows were represented. Several visits to 1 establishment (SCE) were made within each period to obtain the required sample size of about 500 cows/establishment and period.

Given that market dairy cows comprised only a portion of the cattle processed daily at an establishment, samples were obtained from all dairy cows on each day until the number for each sampling period was obtained. Cull dairy cows usually arrived at each establishment in groups, designated as lots. Lots could be completely composed of dairy cattle or, on occasion, composed of cull (nonfed) beef and cull dairy cattle.[7] Most dairy cows included in the study came from auction markets and were identified individually with back tag numbers. Investigators placed a back tag with an unique number on any animal without a back tag. Back tag numbers were used to track the viscera through the slaughter process.

Cecal-colon contents—Cecal-colon contents were obtained for each study cow at the viscera table. For each sample, a single-edged razor blade was used to make a linear incision (7 to 10 cm) at or near the cecal-colon junction, and a tableware plastic spoon was used to retrieve approximately 10 g of contents. A new razor blade and plastic spoon were used for each sample. The contents were placed in an individually identified sterile sampling bag[a] and immediately stored in a cooler chest containing frozen cooler packs. The sampling bags were transferred to another cooler chest in the establishment's walk-in cooler during work breaks at midmorning, noon, midafternoon, and the day's conclusion of sampling. At the end of the sampling day, all samples were shipped next-day delivery in a cooler chest containing frozen cool packs to the Diagnostic Bacteriology Laboratory, **National Veterinary Service Laboratory, USDA-APHIS (NVSL)** in Ames, Iowa, for isolation of *Salmonella* spp.

Isolation technique—All *Salmonella* isolations were conducted at the Diagnostic Bacteriology Laboratory, NVSL, using procedures described[10-13] with minor modifications: a 1-g sample of cecal-colon contents was removed and inoculated into a sterile culture tube with 10 ml of tetrathionate broth to which 0.2 ml of iodine had been added and then incubated at 37 C for 48 hours. After incubation, the tube was vortexed, and 0.1 ml was pipetted into 10 ml Rappaport-Vissiliadis R10 broth and also streaked onto **brilliant green agar plates with novobiocin (BGN)**. The Rappaport tube and BGN plate were incubated at 37 C for 18 to 28 hours. After incubation, the tube was vortexed, and 10 ml was streaked onto **xylose lysine tergitol-4 (XLT-4)** agar and to another BGN plate. Plates were struck for isolation and incubated at 37 C for 18 to 24 hours. Three suspected *Salmonella* colonies were picked from each plate, or all suspected *Salmonella* colonies on the plate were picked if there were fewer than 3 suspected colonies. Each colony picked was transferred to separate tubes containing 5 ml of **triple sugar iron agar (TSI)** and **lysine iron agar (LIA)** slants and incubated at 37 C for 18 to 24 hours. The XLT-4 and BGN plates without presumptive *Salmonella* colonies were incubated at 37 C for an additional 18 to 24 hours and rechecked for suspected colonies. If colonies were found after the additional 24-hour incubation, 3 colonies were picked and differentiated by use of the 2 agar slants described. Any TSI and LIA positive cultures were serotyped at the NVSL, and these results are reported elsewhere.[14]

Statistical analysis—Data were entered into computer spreadsheets[b] and then converted to data sets; all analyses were done by use of statistical software.[15] Tables were constructed to associate prevalence within and between periods and establishments. Prevalence was computed as the number of cows in which *Salmonella* spp were isolated from cecal-colon contents (numerator) divided by the number of cows from which samples were collected for a period and establishment (denominator). Prevalences were compared by use of the test for equal proportions, and the 95% confidence interval for the prevalence was constructed.[15] The level of significance used was $P = 0.05$.

Results

Overall prevalence of *Salmonella* spp was 23.1% **(Table 1)**. The greatest prevalence (54.5%) was at the WE during the summer period; the lowest prevalence (4.3%) was at the CE during the summer period, although a similar prevalence (4.5%) was at the NCE during the winter period. Over both periods, the highest mean prevalence (35.9%) was at the WE, and the lowest (9.1%) was at the NCE. The prevalence for the winter period at the SCE was the sum of 2 visits during that period with prevalences of 2.3% (3/130) and 11.1% (16/144). The prevalence for the summer period at the SCE was the sum of 3 visits with prevalences of 18.6% (49/264), 40.8% (102/250), and 18.1% (28/155).

The proportion of cull cows with *Salmonella* spp was significantly lower during the winter period at the WE, NCE, and SCE (Table 1); the proportion of cattle from which *Salmonella* spp were isolated was significantly greater during the winter period at the SEE and CE. The greatest absolute change (+40.0) in prevalence from the winter to summer periods was found at the WEE; the least change (+9.2) was found at the NCE.

Prevalences of *Salmonella* spp differed significantly among all establishments except the NCE and SCE during the winter period and among all establishments except the SEE and SCE during the summer period (Table 1).

Daily variation in the prevalence of *Salmonella* spp in cecal-colon contents within an establishment was strikingly high **(Table 2)**. As an example, on 1 day at the WE during the summer period, *Salmonella* spp were isolated from samples collected from 93 of 100 cull dairy cows, whereas 2 days later at the same location, *Salmonella* spp were isolated from only 32% of the samples. The range (maximum – minimum daily prevalence) at the WE in the summer period was higher (60.7) than at any other location. Daily variation also was high in the winter at the CE (difference in range values, 46.2). The smallest difference (1.2) of daily variation in prevalence was at the CE during the summer period. The lowest prevalence (0.0%) for any 1 day was at the NCE and SCE during the winter period.

Considering all establishments and sampling periods, some differences were detected for *Salmonella*-infected cull dairy cows slaughtered on particular days of the week **(Table 3)**. The greatest prevalence was in cows slaughtered on Mondays (21.9%) and Tuesdays (28.6%). The lowest mean prevalence was in cows slaughtered on Thursdays (15.8%) and Fridays (11.7%).

Table 1—Prevalence (%) ± margin of error* of *Salmonella* spp in cecal-colon contents collected from cull (market) dairy cattle at 5 slaughter establishments in the United States

	Slaughter establishment (No. of cattle)					
Period†	**WE**	**SEE**	**CE**	**NCE**	**SCE**	**Total**
Winter	14.5 ± 3.1 (504)	37.9 ± 4.2 (525)	27.0 ± 3.9 (508)	4.5 ± 1.8‡ (507)	6.9 ± 3.0‡ (274)	19.5 (2,318)
Summer	54.5 ± 4.0 (582)	27.9 ± 3.9‡ (499)	4.3 ± 1.7 (515)	13.7 ± 3.0 (504)	26.8 ± 3.4‡ (669)	26.2 (2,769)
Total	**35.9 (1,086)**	**33.0 (1,024)**	**15.5 (1,023)**	**9.1 (1,001)**	**21.0 (943)**	**23.1 (5,087)**

*Half the width of a 95% confidence interval: $^{Z\ .025}\sqrt{p\,/\,100(1\text{-}p\,/\,100)100}$ where z is the z distribution. †Winter period includes January and February; summer period includes July, August, and September. ‡Within a row, prevalences that share the same superscript were not found to differ significantly at $P = 0.05$.

WE = West slaughter establishment. SEE = Southeast establishment. CE = Central establishment. NCE = North central establishment. SCE = South central establishment.

Table 2—Daily prevalence (%) of *Salmonella* spp in cecal-colon contents from cull dairy by day on which samples were collected at 5 slaughter establishments across the United States; values in parentheses represent number of cows from which samples were collected

		Slaughter establishment				
Period	**Day**	**W**	**SE**	**C**	**NC**	**SC**
Winter	1	9.6 (73)	20.9 (158)	2.4 (82)	0.0 (69)	4.9 (41)
	2	10.0 (341)	56.0 (291)	48.6 (214)	2.3 (130)	2.6 (39)
	3	35.6 (90)	3.9 (76)	14.6 (212)	13.6 (66)	0.0 (50)
	4	—	—	—	5.7 (105)	13.5 (52)
	5	—	—	—	3.6 (137)	9.8 (92)
Differences in range		**26.0**	**52.1**	**46.2**	**13.6**	**13.5**
Summer	1	32.3 (189)	34.2 (120)	3.9 (102)	7.1 (99)	19.0 (58)
	2	55.6 (293)	31.4 (140)	4.1 (170)	13.7 (51)	7.8 (77)
	3	93.0 (100)	21.8 (179)	4.8 (187)	13.3 (120)	31.8 (85)
	4	—	25.0 (60)	3.6 (56)	20.9 (86)	11.4 (44)
	5	—	—	—	18.7 (75)	17.9 (28)
	6	—	—	—	9.6 (73)	54.8 (124)
	7	—	—	—	—	29.6 (98)
	8	—	—	—	—	22.2 (27)
	9	—	—	—	—	23.0 (87)
	10	—	—	—	—	4.9 (41)
Differences in range		**60.7**	**12.4**	**1.2**	**13.8**	**49.9**

The range (maximum – minimum) of daily *Salmonella* prevalence for each establishment during each period is shown in boldface.
— = *Salmonella* spp not isolated.
See Table 1 for key.

Table 3—Prevalence (%) of *Salmonella* spp in cecal-colon contents from cull dairy cattle by day of the week on which samples were collected at 5 slaughter establishments across the United States

	Slaughter establishment					
Day	**W**	**SE**	**C**	**NC**	**SC**	**Total**
Monday	26.0 (262)	26.6 (278)	3.3 (184)	13.0 (240)	32.6 (310)	21.9[a] (1,274)
Tuesday	31.1 (634)	48.0 (431)	28.9 (384)	6.7 (298)	14.9 (255)	28.6[b] (2,002)
Wednesday	65.8 (190)	16.5 (255)	10.0 (399)	6.2 (387)	20.4 (162)	19.0[c] (1,393)
Thursday	—	25.0 (60)	3.6 (56)	20.9 (86)	12.5 (96)	15.8[cd] (298)
Friday	—	—	—	—	11.7 (120)	11.7[d] (120)
Total	**35.9 (1,086)**	**33.0 (1,024)**	**15.5 (1,023)**	**9.1 (1,011)**	**21.0 (943)**	**23.1 (5,087)**

[a,b,c,d]Prevalences that share the same superscript in the Total column were not found to differ at $P = 0.05$.
See Tables 1 and 2 for key.

Discussion

Because the difference between periods was inconsistent across establishments (period × establishment interaction), it is inappropriate to compare the total prevalence for each establishment with the total prevalence for each of the other establishments. However, statistical comparisons within a period among establishments can be made. Twenty-three of the 25 statistical comparisons made were significant, strongly suggesting that period and establishment affected prevalence of *Salmonella* spp in cull dairy cows.

Each collection period within an establishment spanned only a few days; therefore, the proportion of cows from which *Salmonella* organisms were isolated during a period may not necessarily represent the prevalence rate for each establishment and season over a longer sampling period. To test for seasonal and establishment differences, more visits within each season and over additional years would be required. Other factors likely contributed to the period differences detect-

ed, such as a change in the source of cows, the distance cattle traveled to an establishment, cleanliness of transport, the cattle handling behaviors of slaughter establishment personnel, the nature of sanitation in establishment holding pens, the time cattle were held in establishment pens, and the degree of implementation of quality assurance programs within an establishment.

Depending on site and season, the prevalence of *Salmonella* spp in healthy market dairy cows at slaughter was considered to be high. Overall prevalence was 23.1% (with a range accounting for location and season between 4.3 and 54.5%). These prevalence values were far greater than that (0.46%) found in another study[16] of healthy cull dairy cows where fecal swabs and mesenteric lymph nodes were subjected to bacteriologic culture for *Salmonella* spp. Grau and Brownlie,[17] examining rumen fluid obtained from cattle after slaughter, reported that of 193 animals tested, 87 (45%) were infected with *Salmonella* spp. Similarly, Samuel et al[18] isolated *Salmonella* spp from rumen contents of 62% of slaughtered cattle. Wray and Sojka,[19] citing various sources, indicated that the incidence of *Salmonella* spp in cattle at slaughter varied between 0.3 and 11.6%. Those investigators also pointed out that despite a number of surveys examining the prevalence of *Salmonella* spp in cattle at slaughter, comparisons of results are difficult because of variation in specimens examined and sampling techniques used. In the study reported here, a standard protocol for sampling was used at each establishment, a common specimen (cecal-colon content) was obtained, and a single laboratory using an established isolation protocol conducted the *Salmonella* isolations. Another finding of our study was the wide daily variation in prevalence, 0.0 to 93%. This variation indicates that a survey for prevalence of *Salmonella* spp in cattle at any location must span several days to more accurately reflect prevalence. Additionally, this variation suggests that the proportion of cattle with *Salmonella* spp entering a slaughter establishment is likely determined by the source of the cattle. Therefore, measures to reduce *Salmonella* spp load at the dairy farm and during transport to slaughter should reduce the risk of accumulation and spread within a slaughter establishment. Efforts to reduce *Salmonella* organisms before and during slaughter may need to be increased during particular seasons and within geographic regions. The day-to-day variation, variation by day of the week, variation by season, and variation by region in the prevalence of *Salmonella* could not have been determined without repeated sampling. More visits to the slaughter establishments participating in this study and sampling at those locations for a longer duration would have improved our estimates of prevalence and the variation in prevalence. Additionally, studies are needed that examine the prevalence of *Salmonella* spp in cull dairy cows on the farm, at auction markets, and then at slaughter.

Because bacterial culture can fail to detect *Salmonella* spp in some contaminated samples and the procedure is less likely to result in a false-positive result, the prevalence of *Salmonella* spp in market dairy cattle at slaughter may be higher than reported here. If so, there is urgency in improving on-farm and cattle transport sanitation practices and in developing improved diagnostic tests for the rapid detection of food-borne pathogens such as *Salmonella* spp. The prevalences found in this study of *Salmonella* spp in healthy cull dairy cows at slaughter require optimal Hazard Analysis Critical Control Point (HACCP) programs in slaughter establishments to minimize the risk of contamination of carcasses with *Salmonella* organisms.

[a]Whirl-Pak, Nasco, Fort Atkinson, Wis.
[b]Microsoft Corp, Redmond, Wash.

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