

Comparison of Individual, Pooled, and Composite Fecal Sampling Methods for Detection of *Salmonella* on U.S. Dairy Operations

J. E. LOMBARD,^{1*} A. L. BEAM,¹ E. M. NIFONG,¹ C. P. FOSSLER,¹ C. A. KOPRAL,¹ D. A. DARGATZ,¹ B. A. WAGNER,¹ M. M. ERDMAN,² AND P. J. FEDORKA-CRAY³

¹U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, 2150 Centre Avenue, Building B, Fort Collins, Colorado 80526-8117; ²U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, 1800 Dayton Avenue, Ames, Iowa 50010; and ³U.S. Department of Agriculture, Agricultural Research Service, Russell Research Center, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, 950 College Station Road, Athens, Georgia 30604, USA

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ABSTRACT

The objectives of this study were to estimate the prevalence of *Salmonella* for individual, pooled, and composite fecal samples and to compare culture results from each sample type for determining herd *Salmonella* infection status and identifying *Salmonella* serovar(s). During the U.S. Department of Agriculture National Animal Health Monitoring System Dairy 2007 study, data and samples were collected from dairy operations in 17 major dairy states. As part of the study, composite fecal samples (six per operation) were collected from cow areas, such as holding pens, alleyways, and lagoons, where manure accumulates. Fecal samples also were collected from individual cows (35 per operation), and fecal sample pools were created by combining samples from 5 cows (7 per operation). A total of 1,541 composite fecal samples were collected from 260 operations in 17 states, and 406 (26.3%) of these samples were culture positive for *Salmonella*. Among the 116 operations for which all three sample types were obtained, 41.4% (48 operations) were *Salmonella* culture positive based on individual samples, 39.7% (46 operations) were positive based on pooled samples, and 49.1% (57 operations) were positive based on composite fecal samples. Relative to individual samples, the sensitivity of composite fecal samples for determining herd infection status was 85.4% and the sensitivity of pooled fecal samples was 91.7%. On 33.6% of operations (39 of 116), *Salmonella* was cultured from all three fecal sample types (individual, pooled, and composite), and 20 (51.3%) of these operations had exactly the same serovar in all three sample types. Use of composite fecal samples is less costly and time-consuming than use of individual or pooled samples and provides similar results for detecting the presence and identifying serovars of *Salmonella* in dairy herds. Therefore, composite sampling may be an appropriate alternative to culture of individual samples when assessing *Salmonella* status in dairy herds.

Salmonellosis is the second most common food-related illness in the United States but is the leading cause of hospitalization and death attributed to foodborne transmission of disease agents (19). The estimated annual cost due to *Salmonella* infections is \$11.4 billion, including medical expenses, lost productivity, and premature deaths (20). The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service monitors and regulates meat and poultry processing facilities, and product contamination with *Salmonella* is routinely monitored to assess the effectiveness of pathogen reduction strategies (i.e., hazard analysis and critical control point procedures) used in these facilities.

Salmonella infection in cattle can result in clinical disease and occasionally leads to death; however, most *Salmonella* infections in bovids are subclinical, and shedding of *Salmonella* cells can continue for extended periods (10). The USDA National Animal Health Monitoring System (NAHMS) Dairy 2002 study included culturing of

individual fecal samples from dairy cows on 97 operations in 21 states (4). Results indicated that 7.3% of cows were shedding *Salmonella* in their feces, and 30.9% of dairy operations had at least one animal shedding *Salmonella*. Animals without clinical signs of *Salmonella* infection can shed the organism in feces and into the environment (10, 17). The presence of *Salmonella* creates a potential risk for human exposure to *Salmonella* through direct contact with cattle and their feces (12) or through consumption of unpasteurized milk and contaminated meat or produce (8, 25).

Culturing of individual cow fecal samples is used for detection of *Salmonella* on cattle operations and is the most common method for assessing individual cow and herd *Salmonella* status. However, this method is expensive and time-consuming (26). In previous studies, culture of pooled fecal samples from individual cows has been evaluated as a tool for screening herds for *Salmonella* and other bacterial pathogens (14, 21) that require samples collected from individual cows. Evaluation of pooled fecal culturing for detection of *Mycobacterium avium* subsp. *paratuberculosis*

* Author for correspondence. Tel: 970-494-7245; Fax: 970-494-7228; E-mail: jason.e.lombard@aphis.usda.gov.

revealed that the sensitivity of the method can vary based on the stage of the disease and the shedding level of animals tested (29).

Samples collected from animal environments are being used in a variety of animal housing systems, including pet shelters, veterinary hospitals, and swine and turkey operations, to determine the presence of *Salmonella* (1, 2, 5, 22). Composite fecal sampling also has been used successfully to identify dairy herds infected with *M. avium* subsp. *paratuberculosis* (3, 16, 18). Composite fecal samples are typically collected from areas on dairy operations where manure accumulates from a majority of adult animals, such as holding pens, alleyways, and lagoons. Van Kessel et al. (25) studied one *Salmonella*-infected dairy herd and found that 60 to 100% of the four or five composite fecal samples collected at one time were culture positive, and overall more than 90% of composite fecal samples were positive for *Salmonella* over a 3-year period. *Salmonella* was also isolated from inline milk filters that were examined weekly in this same herd.

The objectives of this study were to estimate the prevalence of *Salmonella* for individual, pooled, and composite fecal samples and to compare the effectiveness of culturing individual cow fecal samples, pooled fecal samples, and composite fecal samples for determining herd *Salmonella* infection status and identifying the *Salmonella* serovar(s).

MATERIALS AND METHODS

Study design. Data were collected during the NAHMS Dairy 2007 study from dairy operations in 17 major dairy states, which represented 79.5% of U.S. dairy operations and 82.5% of the dairy cow population. The survey design was a stratified random sample with unequal selection probabilities across strata to ensure that large dairy operations were well represented in the sample. Two regions were included in the study. The west region included California, Idaho, New Mexico, Texas, and Washington, and the east region included Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin. Additional details regarding the design of the survey can be found elsewhere (24). The number of herds to be included in the *Salmonella* component of the study was based on an expected *Salmonella* herd-level prevalence of $50\% \pm 6\%$ (with a confidence of 95%) and on laboratory capacity. Each participating state was allocated a number of herds to sample for *Salmonella* testing based on the number of respondent operations within the state. Herds were selected within a state based on their willingness to participate and were required to have 30 or more dairy cows.

Individual and pooled fecal sample collection. A convenience sample of 116 dairy operations where composite fecal samples were collected also took part in the collection of individual fecal samples. On five additional operations, individual and pooled samples were collected but composite fecal samples were not. A total of 121 operations provided both individual and pooled sample results. The targeted number of individual cow samples collected from each operation was 35. Samples were collected by state and federal animal health personnel between February and August 2007. Thirty-five individual cow samples were collected from each operation, with guidelines to collect up to five samples from sick cows and up to five samples from cows to be culled within 7 days. At least 25 of the 35 samples had to come from cows with saleable

milk. No additional information (lactation, milk production, etc.) was collected on the individual cows sampled.

An approximately 100-g (golf-ball size) sample of manure was obtained with a single-use glove via rectal retrieval from each cow. Upon arrival at the laboratory, individual samples were aliquoted; one portion of the sample was used as an individual sample, and another portion was combined with other individual samples from the same operation to form a pooled sample. Each individual cow sample was equally represented in the respective pooled sample. The individual samples from a single operation were allocated into up to seven pools (five individual samples per pool) based on sample collection order regardless of whether the pool may have included sick or cull cows. When the number of individual samples collected was not a multiple of five, the final pool that was created contained individual samples from fewer than five cows. When one or more of the individual or pooled samples from an operation was culture positive, the operation was classified as culture positive for *Salmonella* for the respective sample type.

Composite fecal sample collection. A convenience sample of 260 dairy operations took part in the collection of composite fecal samples, and 116 of these operations also collected individual and pooled samples for *Salmonella* testing. Because another objective of the NAHMS Dairy 2007 study was to estimate herd-level prevalence of *M. avium* subsp. *paratuberculosis*, the number of samples collected from each operation ($n = 6$) was based on the guidelines from the *Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program* (23). Samples were collected by state and federal animal health personnel between February and August 2007.

Composite fecal samples were taken from six areas at each operation where manure from adult cows accumulated. Recommended locations for sampling included but were not limited to common pens or alleyways, manure pit or other manure storage area, holding pens or exit ways from the milking parlor, gutter cleaners, and manure spreaders. For each composite sample, approximately 120 g of manure and/or slurry was taken from each of six sites within the respective area and combined to form a single composite sample of approximately 720 g. When one or more of the six composite fecal samples from an operation was culture positive, the operation was classified as culture positive for *Salmonella*. When none of the six samples was culture positive, the operation was classified as culture negative.

Shipping and culture. Individual and composite fecal samples were shipped overnight on ice to the USDA Agricultural Research Service Bacterial Epidemiology and Antimicrobial Resistance Research Unit (Athens, GA) for culture. Culture of individual samples was conducted according to methods previously described (28). All cultures were performed on fresh, nonfrozen samples. Approximately 1 g of feces from each sample was placed into two enrichment media (gram-negative Hajna broth and tetrathionate broth) and incubated at 37°C for 24 and 48 h, respectively. Aliquots (100 μ l) from each broth culture were transferred into Rappaport R-10 medium for a secondary enrichment, incubated overnight at 37°C, and then streaked onto brilliant green agar with sulfadiazine and xylosine lysine Tergitol 4 plates. Plates were incubated overnight at 37°C. Up to four colonies with the typical appearance of *Salmonella* from each plate were inoculated into triple sugar iron and lysine iron agar slants and incubated overnight at 37°C. Isolates presumed to be *Salmonella* were identified to serogroup using serogroup-specific sera, and isolates of different serogroups from each sample were kept. When all four colonies from a sample belonged to the same serogroup, only

TABLE 1. Number and percentage of individual, pooled, and composite fecal samples from U.S. dairies that were culture positive for *Salmonella* by herd size, region, and season of collection

Sample type and result	Herd size ^a			Region		Season ^b			Total
	Small	Medium	Large	West	East	Winter	Spring	Summer	
Individual fecal samples (121 operations)									
No. positive	73	283	225	27	554	4	343	234	581
Total tested	1,245	1,629	1,290	700	3,464	442	2,396	1,326	4,164
% positive	5.9	17.4	17.4	3.9	16.0	0.9	14.3	17.6	14.0
<i>P</i> value ^c		0.0035		0.0012			0.0004		
Pooled fecal samples (121 operations)									
No. positive	25	96	71	13	179	3	113	76	192
Total tested	251	327	259	140	697	90	480	267	837
% positive	10.0	29.4	27.4	9.3	25.7	3.3	23.5	28.5	22.9
<i>P</i> value		0.0006		0.001			0.0003		
Composite fecal samples (260 operations)									
No. positive	102	151	153	36	370	48	231	127	406
Total tested	541	551	449	311	1,230	195	910	436	1,541
% positive	18.9	27.4	34.1	11.6	30.1	24.6	25.4	29.1	26.3
<i>P</i> value		<0.0001		<0.0001			0.5421		
All samples (265 operations)									
No. positive	200	530	449	76	1,103	55	687	437	1,179
Total tested	2,037	2,507	1,998	1,151	5,391	727	3,786	2,029	6,542
% positive	9.8	21.1	22.5	6.6	20.5	7.6	18.1	21.5	18.0

^a Herd sizes: small, <100 cows; medium, 100 to 499 cows; large, ≥500 cows.

^b Seasons: winter, February and March; spring, April through June; summer, July and August.

^c *P* values were adjusted for herd size, region, and season of sample collection.

one isolate was retained. Isolates were subsequently identified to serotype at the National Veterinary Services Laboratories (Ames, IA) using previously described methods (7). Antigenic formulae for somatic (O) and flagellar (H) antigens were used to determine serovar (11). When O or H antigens could not be determined, the isolate was reported as rough or nonmotile, respectively.

Data analysis. Descriptive statistics and hypothesis tests were produced using SAS (version 9.2, SAS Institute Inc., Cary, NC). Participating herds were categorized into three herd-size groups based on adult cow inventory on 1 January 2007: small = 30 to 99 cows, medium = 100 to 499 cows, and large = 500 or more cows. Samples also were categorized based on season of collection: winter = February and March, spring = April through June, and summer = July and August. Agreement between sampling methods was evaluated using the kappa statistic and McNemar's test with PROC FREQ in SAS. The effects of herd size, region, and season on the *Salmonella* status of individual, pooled, and composite fecal samples were evaluated using PROC GENMOD in SAS, which utilizes a generalized estimating equations approach to account for correlations between samples within a farm (Table 1). Correlations for operation-level *Salmonella* status between the three sampling methods were determined by specifying Spearman in the PROC CORR procedure of SAS. *P* values of <0.05 were considered significant. To assess the effect of varying numbers of environmental composite samples on the operation-level *Salmonella* status, random numbers were generated in SAS, and samples were sorted randomly within each operation. The first *n* samples (where *n* = 1, 2, 3, 4, or 5) from each farm were selected. Different random numbers were generated for each repetition. All 260 operations with environmental composite samples were included in this analysis.

RESULTS

Sample level: individual and pooled fecal samples.

Fourteen percent (581 of 4,164) of the individual fecal samples from 121 dairy operations were culture positive for *Salmonella*, and 22.9% (192 of 837) of the pooled fecal samples (from the same 121 operations) were also culture positive for *Salmonella* (Table 1). Table 1 shows associations between sample-level results and descriptive factors for the operation and sampling type.

A significant association (*P* = 0.004) was found between herd size and individual fecal culture result after accounting for region and season of collection with PROC GENMOD. Small herds had a lower percentage of culture-positive individual fecal samples (5.9%) compared with medium and large herds (17.4 and 17.4%, respectively) (Table 1). A significant association between herd size and pooled fecal culture results (*P* < 0.0001) also was found. Small herds had the lowest percentage of culture-positive pooled fecal samples (10.0%), whereas medium herds had the highest percentage (29.4%).

Individual and pooled samples collected from the east region were more likely to be culture positive for *Salmonella* (16.0 and 25.7%, respectively) than were those collected from the west region (3.9 and 9.3%, respectively) (*P* = 0.001 and 0.001, respectively) (Table 1).

Individual samples collected in spring and summer were more likely to be culture positive for *Salmonella* (14.3 and 17.6%, respectively) than were samples collected in winter (0.9%) (*P* < 0.001). The same seasonal differences

TABLE 2. Top seven *Salmonella* serovars identified by culture of individual, pooled, and composite fecal samples from U.S. dairies

Fecal sample type	<i>Salmonella</i> serovar	No. (%) of isolates	No. of operations
Individual	Cerro	167 (26.9)	14
	Kentucky	137 (22.1)	14
	Montevideo	72 (11.6)	6
	Meleagridis	58 (9.4)	6
	Mbandaka	50 (8.1)	3
	Derby	29 (4.7)	1
	Muenster	22 (3.5)	8
	Other	85 (13.7)	32
Total		620	
Pooled	Cerro	49 (24.1)	12
	Kentucky	46 (22.7)	14
	Meleagridis	24 (11.8)	6
	Montevideo	17 (8.4)	7
	Mbandaka	15 (7.4)	3
	Muenster	11 (5.4)	5
	Seftenberg	8 (3.9)	3
	Other	33 (16.3)	21
Total		203	
Composite	Cerro	114 (24.8)	26
	Kentucky	70 (15.3)	21
	Montevideo	43 (9.4)	13
	Meleagridis	39 (8.5)	10
	Muenster	29 (6.3)	12
	Anatum	23 (5.0)	8
	Mbandaka	22 (4.8)	9
	Other	119 (25.9)	67
Total		459	

also were observed for pooled samples, where 23.5 and 28.5% were positive in spring and summer, respectively, compared with 3.3% in winter ($P < 0.001$) (Table 1).

From the 581 culture-positive individual samples, 620 isolates were recovered (Table 2). Twenty-five unique serovars were represented (21 named serovars and 4 antigenic formula serovars). *Salmonella enterica* subsp. *enterica* serovars Cerro and Kentucky were represented by 26.9

and 22.1% of the isolates recovered, respectively, and were each recovered on 14 operations.

Two hundred three isolates were recovered from the 192 culture-positive pooled samples, and 21 unique serovars were identified. Two of the 21 were antigenic serovars. *Salmonella* serovars Cerro and Kentucky were represented by 24.1 and 22.7% of the isolates recovered, respectively, and were isolated from 12 and 14 operations, respectively.

Sample level: composite fecal samples. A total of 1,541 composite fecal samples were collected from 260 operations; and of these samples, 406 (26.3%) were culture positive for *Salmonella* (Table 1).

A significant association was observed between herd size and composite fecal culture results at the sample level ($P < 0.0001$) after accounting for region and season of collection. Small herds had the lowest percentage of culture-positive composite fecal samples (18.9%), whereas large herds had the highest percentage (34.1%) (Table 1).

As was observed for individual animal and pooled samples, a higher percentage of composite fecal samples collected in the east region were culture positive for *Salmonella* (30.1%) compared with the west region (11.6%) (Table 1).

In contrast to the results of the individual and pooled samples, there was no difference in the percentage of composite fecal samples that were culture positive for *Salmonella* by season ($P = 0.5$) (Table 1).

Common pens and alleyways were the most common locations for collection of composite fecal samples (377 and 321 samples, respectively), and flush water and manure spreader samples were the least common (23 and 45 samples, respectively) (Table 3). The percentage of composite fecal samples that were culture positive for *Salmonella* was similar across collection locations: flush water (39.1%), floor of holding pen (32.8%), common alleyway (29.3%), exit way from parlor (28.6%), gutter cleaner (28.0%), lagoon (26.5%), manure pit (25.3%), common pen (23.6%), and manure spreader (11.1%). There was no significant association between composite fecal sample source and

TABLE 3. Source, number, and percentage of U.S. dairy composite fecal samples and operations that were culture positive for *Salmonella*

Sample source	Samples			Operations		
	No. positive	Total tested	% positive	No. positive	Total tested	% positive
Flush water	9	23	39.1	9	23	39.1
Floor of holding pen	44	134	32.8	41	126	32.5
Common alleyway	94	321	29.3	64	201	31.8
Exit way from parlor	40	140	28.6	35	133	26.3
Gutter cleaner	30	107	28.0	17	56	30.4
Lagoon	18	68	26.5	17	67	25.4
Manure pit	24	95	25.3	24	86	27.9
Common pen (e.g., loafing barn, drylot)	89	377	23.6	50	190	26.3
Manure spreader	5	45	11.1	5	42	11.9
Other	53	231	22.9	38	140	27.1
Total	406	1,541	26.3	114	260	43.8

TABLE 4. Comparison of individual, pooled, and composite fecal culture results for herd-level detection of *Salmonella* on U.S. dairies

Fecal sample <i>Salmonella</i> results			Operations	
Individual	Pooled	Composite	No.	%
+	+	+	39	33.6
-	-	+	15	12.9
+	+	-	5	4.3
+	-	-	2	1.7
+	-	+	2	1.7
-	+	+	1	0.9
-	+	-	1	0.9
-	-	-	51	44.0
Total			116	100.0

Salmonella culture result ($P = 0.09$) at the sample level. However, when sample locations were analyzed and adjusted for clustering within an operation, manure spreaders were significantly less likely to be *Salmonella* positive than were all other sample locations with the exception of the common pen ($P = 0.05$).

From 406 culture-positive composite fecal samples, 459 isolates were recovered (Table 2). Forty-three unique types were identified, comprising 30 serovars and 13 partial antigenic formulae that could not be identified to serovars; 74.1% of isolates belonged to seven serovars (Table 2). *Salmonella* serovars Cerro and Kentucky were represented by 24.8 and 15.3% of the isolates recovered, respectively. *Salmonella* Newport was represented by 3.1% of the isolates recovered. In 26 operations, *Salmonella* Cerro was recovered from composite fecal samples, and 21 operations had *Salmonella* Kentucky.

Comparison of individual, pooled, and composite fecal samples. All three sample types were collected and submitted from 116 operations in 17 states, for a total of 5,483 samples. Of these, 3,990 samples were from individual cows (approximately 35 cows per herd). Fewer than 35 individual samples were collected and submitted from 13 of the 116 operations (11.2%). From the individual cow fecal samples, 802 pooled fecal samples were created, and 110 of the 116 operations submitted 7 pooled samples. The remaining 691 samples were composite fecal samples from common cow areas at each dairy operation; 113 of the 116 operations (97.4%) submitted the requested six samples. One operation submitted five composite samples, and two operations submitted four composite samples.

Of the 116 operations where all three sample types were collected, 48 operations (41.4%) were classified as culture positive for *Salmonella* based on results from individual samples, 46 (39.7%) were positive based on pooled samples, and 57 (49.1%) were positive based on composite samples (Table 4). Of the 48 culture-positive operations found via individual fecal sampling, pooled fecal sampling identified 44 (91.7%) and composite fecal sampling identified 41 (85.4%) as positive. Sixteen operations were

culture positive based on composite fecal culture results but were not *Salmonella* positive based on individual fecal sampling. Fifty-one operations (44.0%) were culture negative by all three sampling methods, and 39 operations (33.6%) were culture positive by all three sampling methods.

A comparison of individual and pooled sample results used to identify operations as culture positive or negative revealed almost perfect agreement between the two sampling methods ($\kappa = 0.893$) (Table 5). The kappa values for comparing herd-level results for individual or pooled sampling with results from composite sampling were 0.602 and 0.602, respectively, suggesting moderate to substantial agreement. Based on McNemar's test for correlated proportions, there were no differences between herd-level individual sample and pooled sample culture results and between herd-level individual sample and composite sample culture results ($P = 0.41$ and 0.06 , respectively). However, there was a significant difference in proportions of culture-positive operations when comparing pooled (39.7%) and composite (49.1%) samples ($P = 0.02$).

Operation-level prevalence for all three sample types was significantly correlated ($P < 0.0001$) based on the Spearman rank correlation. Relative to individual samples, the sensitivity of composite fecal sampling for determining herd infection status was 85.4% and the sensitivity of pooled fecal sampling was 91.7%. A comparison of herd-level individual cow prevalence and herd-level composite prevalence is presented in Figure 1. With the exception of a single operation with an individual animal prevalence of 25.7%, composite fecal samples consistently detected infected operations when the individual cow prevalence was above approximately 6%. When composite fecal samples were used as the standard, the sensitivities of individual and pooled sampling for determining herd status were 71.9 and 70.2%, respectively.

The within-herd prevalence was estimated based on the number of culture-positive individual cow samples divided by the total number of individual cow samples cultured. Overall, pooled samples detected 91.7% of herds identified as *Salmonella* positive via individual cow sampling (Table 6). For herds with more than 10% prevalence, all herds were detected by pooled fecal sampling, and 81% of herds with 10% or lower prevalence were detected by pooled sampling. Composite fecal sampling detected 71.4% of herds with 10% or lower prevalence, 91.7% of herds with 10 to 50% prevalence, and 100% of herds with higher than 50% prevalence. Composite fecal sampling also detected 15 additional operations that were *Salmonella* negative by individual and pooled sampling (Table 4).

At the operation level, differences in *Salmonella* prevalence by herd size were observed for all three sampling methods; a lower percentage of small operations were culture positive compared with medium and large operations ($P < 0.001$) (Table 7).

The percentage of operations that were culture positive was higher in the east region than in the west region for all sampling methods.

TABLE 5. Operation-level agreement of individual, pooled, and composite fecal samples collected for *Salmonella* culture from 116 U.S. dairies

Sampling method	Reference method	Herd-level sensitivity (95% CI)	Cohen's kappa (95% CI)	McNemar <i>P</i> value	Efficiency (95% CI)	Proportion agreement		
						Overall	Positive	Negative
Pool	Individual	91.7 (80.0–97.7)	0.8927 (0.8092–0.9762)	0.4142	94.8 (89.1–99.6)	0.9483	0.9362	0.9565
Composite	Individual	85.4 (72.2–93.9)	0.6023 (0.4585–0.7460)	0.0606	80.2 (71.8–87.0)	0.8017	0.7810	0.8189
Composite	Pool	87.0 (73.7–95.1)	0.6020 (0.4591–0.7450)	0.0218	80.2 (71.8–87.0)	0.8017	0.7767	0.8271
Individual	Composite	71.9 (58.5–83.0)	0.6023 (0.4585–0.7460)	0.0606	80.2 (71.8–87.0)	0.8017	0.7810	0.8189
Pool	Composite	70.2 (56.6–81.6)	0.6020 (0.4591–0.7450)	0.0218	80.2 (71.8–87.0)	0.8017	0.7767	0.8217

Of the 116 operations that collected all three sample types, on 14 operations (12.1%) the composite fecal samples and the individual and pooled samples were collected in different seasons. For operations classified as culture positive based on individual or pooled samples, the prevalence in winter was lower than that in spring or summer. However, there were no differences by season in percentage of operations that were culture positive based on composite fecal samples ($P = 0.4$).

Salmonella was recovered from all three sample types on 39 operations. Of these, 20 operations (51.3%) had exact serovar matches among all three sample types, and 14 operations (35.9%) had at least one of the serovars recovered from at least two of the three sample types. Thirteen of the herds with exact serovar matches had one serovar present, six herds had two serovars present, and one herd had three serovars present. The highest number of serovars was detected with composite fecal sampling (74 serovars), followed by individual cow sampling (66 serovars), and then pooled fecal samples (57 serovars).

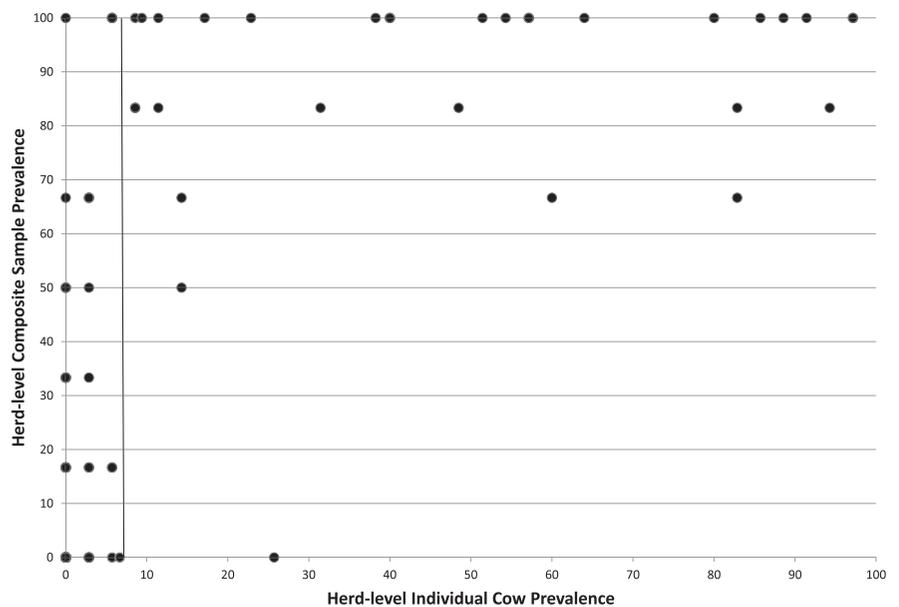
In comparison to the individual fecal sampling methods used in this study, composite fecal sampling performed well for identifying serovars present on the operation. Among the 50 operations on which *Salmonella* was identified by individual or pooled sampling methods, composite fecal sampling identified all serovars found by individual sampling on 28 operations (56%) and some but not all of the same serovars on 6 operations (12%) (excluding isolates with unnamed antigenic formulas). Composite fecal sampling also identified the predominant serovar in the greatest number of cattle from individual sampling on 35 operations. Among these 35 operations, pooled fecal sampling identified all serovars found by individual sampling on 27 operations (79.1%) and some but not all of the same serovars on 8 operations (22.9%). Composite fecal sampling revealed a higher level of diversity of serovars compared with the other sampling methods. Among the 50 operations on which *Salmonella* was identified by individual or pooled and composite sampling, 79 serovars per operation were found by composite fecal sampling, 66 were found by individual fecal sampling, and 77 were found by pooled fecal sampling.

DISCUSSION

The objectives of this study were to estimate the prevalence of *Salmonella* for individual, pooled, and composite fecal samples and to compare the results of culturing individual cow fecal samples, pooled fecal samples, and composite fecal samples for determining herd *Salmonella* infection status and identifying *Salmonella* serovars.

Although a convenience sample was utilized, the 265 operations that were included in this study are believed to reflect the diversity of management practices on U.S. dairy operations across herd sizes and between regions. All cultures were performed on fresh samples at a single laboratory using standardized methods that permitted direct comparison of study results among the sampling methods and herds represented within the study. For the sampling method

FIGURE 1. Comparison of herd-level *Salmonella* prevalence based on individual cow fecal samples and composite fecal samples for 116 dairy operations (vertical line represents the herd-level prevalence of approximately 7% based on individual cow samples).



comparison, only the 116 operations that used all three sampling methods were included.

In previous studies, large operations have been more likely than small operations to be *Salmonella* positive (9, 28). Large herds in those two studies were defined as 100 cows or more, which would have been considered medium herds in the present study. In the present study, a higher percentage of large herds were culture positive compared with small herds, regardless of the sample type. Because herd size is typically correlated with many aspects of management (milking facilities, feed types, region of the United States, etc.), this measure is generally included as a confounding factor when evaluating variables such as season and region. Herd size does not provide useful information for assisting the industry in lowering the prevalence of *Salmonella* but is important when evaluating associations with other variables.

The higher *Salmonella* herd-level prevalence in the east region compared with the west region was unexpected. In the NAHMS Dairy 2002 study (4), the highest prevalence was in the south region and then the west region followed by the midwest and northeast regions. When results from the midwest, northeast, and southeast regions were collapsed into a single region—as was essentially done when designing the 2007 study—the prevalence in the west was almost twice that of the combined regions for the east (42.9 versus 26.1%). Because this difference was found with both individual and pooled samples, the data appear to reflect differences in

prevalence in those regions at the time of the study. Although temperature and moisture data were not collected in this study, differences in these conditions for these regions during the sample collection period may have accounted for the difference in regional prevalence. Differences in types of operations sampled

TABLE 7. Number and percentage of operations testing culture positive for *Salmonella* by individual, pooled, and composite fecal samples by herd size, region, and season of collection for 116 U.S. dairy operations that collected all three sample types

Fecal sample type and result	Herd size ^a			Region		Season ^b			Total
	Small	Medium	Large	West	East	Winter	Spring	Summer	
Total no. of operations	36	45	35	19	97	13	68	35	116
Individual									
No. positive	8	22	18	5	43	2	27	19	48
% positive	22.2	48.9	51.4	26.3	44.3	15.4	39.7	54.3	41.4
<i>P</i> value ^c		0.0004		0.0074		0.0157			
Pooled									
No. positive	6	21	19	6	40	2	26	18	46
% positive	16.7	46.7	54.3	31.6	41.2	15.4	38.2	51.4	39.7
<i>P</i> value		<0.0001		0.0243		0.0193			
No. of operations ^d						21	67	28	116
Composite									
No. positive	9	24	24	7	50	8	36	13	57
% positive	25.0	53.3	68.6	36.8	51.5	38.1	53.7	46.4	49.1
<i>P</i> value		<0.0001		0.0020		0.4187			
All sampling methods									
No. positive	12	27	26	9	56				
% positive	33.3	60.0	74.3	47.4	57.7				
<i>P</i> value		<0.0001		0.0121					

^a Herd sizes: small, <100 cows; medium, 100 to 499 cows; large, \geq 500 cows.

^b Seasons: winter, February and March; spring, April through June; summer, July and August.

^c *P* values were adjusted for herd size, region, and season of sample collection.

^d On 14 of the 116 operations, individual and pooled samples were collected during a different season than when the composite fecal samples were collected.

of *Salmonella* surviving or being less stressed (and therefore easier to culture). Another possible reason for the discrepancy in the results of the different sample types is that the individual cows sampled might not have been a random sample and therefore were not representative of the entire herd. Composite fecal samples appear not to be impacted by season and thus may be a better monitoring tool during the winter, when individual animal sampling indicates a lower *Salmonella* prevalence than found other seasons.

Previous research designed to test for the presence of *Salmonella* in herds has often involved culture of individual fecal samples from a representative number of cows. This process is costly and labor intensive, and as herd size increases or within-herd prevalence decreases, additional individual cow samples must be collected to maintain a similar herd sensitivity and accurately determine the herd infection status. One alternative to individual animal sampling is targeted sampling. According to one study (27), cows near the time of calving were most likely to be shedding *Salmonella* in the feces. However, in that study the cattle group with the highest prevalence of positive samples differed widely among herds, making it difficult to select target animals for culture that would be appropriate for all herds. Another option for decreasing the cost of herd *Salmonella* testing is pooling individual fecal samples. A comparison of results for pooled sample cultures with results from individual samples revealed strong agreement for detection of *Salmonella* when the number of individual

samples per pool was 20 or fewer (15). However, with pooled sampling, individual fecal samples still must be collected, and creating the pools is a substantial time and labor burden for the laboratory. Composite fecal sampling is the collection of feces from areas where manure accumulates and should represent a larger number of cattle compared with individual sampling. While this approach may help avoid the effect of variations in *Salmonella* shedding in individual animals, it may impact survival of *Salmonella* from the environment; sampling areas were not equal in terms of number of *Salmonella*-positive samples. Collection and culture of six composite fecal samples is both less costly and less time-consuming than collecting and culturing 35 to 40 fecal samples from individual cows.

The number of composite fecal samples used in this study was based on guidelines from the Voluntary Bovine Johne's Disease Control Program (23). Although we could not infer the sensitivity that would be obtained when culturing more than six samples, the effect of culturing fewer samples was explored. The proportions of the 260 operations detected as *Salmonella* positive based on one, two, three, four, five, and six composite fecal samples were 23.1, 33.1, 38.1, 38.9, 41.2, and 43.8%, respectively.

This study is the first broad national comparison of composite fecal sampling, individual fecal sampling, and pooled fecal sampling conducted to determine the effectiveness of each method for detecting the presence of *Salmonella* in dairy herds. The results of this study indicate

that composite fecal sampling of areas such as alleyways, common pens, holding pens, milking parlor exits, and lagoons or manure pits provides results (i.e., sensitivity) comparable to those obtained from individual and pooled sampling for determining herd *Salmonella* status and identifying the associated *Salmonella* serovars.

In the present study, a slightly higher percentage of *Salmonella*-positive operations was detected via the evaluation of composite fecal samples than via the evaluation of individual and pooled samples, although this difference was not statistically significant. This finding may indicate that composite fecal sampling is more sensitive at the sample level than the other two sampling methods, primarily because of the increased number of cattle sampled indirectly through composite sampling; however, some composite fecal samples may have tested positive because of *Salmonella* from sources other than dairy cattle, such as wildlife and human movement within an operation. *Salmonella* has been cultured from the feces of various species of wildlife (13). Nevertheless, in the present study the composite fecal samples collected (except those from manure storage systems) contained fecal material that had mostly been deposited within the previous 24 h, depending on cleaning schedule and source of samples, minimizing the chances that the *Salmonella* isolates were from different host species. Because most of the *Salmonella* isolates identified in this study are commonly recovered from cattle and because the predominant serovars recovered with each of the three sampling methods were similar, the majority of the *Salmonella* isolates detected were thought to be from the dairy cows.

This study also is the first national comparison of serovars identified from operations based on individual cow fecal samples, pooled fecal samples, and composite fecal samples. Culture of *Salmonella* is expensive, and previous research focused on *Salmonella* testing of cattle on the farm typically has involved a sample size designed to detect the presence of *Salmonella*, not to estimate within-herd prevalences. This same type of design was used in the present study. To identify all serovars present on an operation at a point in time and to determine the predominant serovar would have required more samples and/or isolates selected per operation than were collected and selected in this study. Thus, the serovar comparison should be interpreted in light of how well composite fecal sampling and pooled fecal sampling compare with the individual fecal sampling methods typically used in research studies examining *Salmonella* presence in dairy cattle.

Salmonella Cerro and *Salmonella* Kentucky were the most commonly identified serovars in all three sample types. These serovars were also the most commonly reported in a New York study, and *Salmonella* Cerro is becoming more prevalent (6). *Salmonella* Newport, which can cause clinical disease in cattle and humans, had relatively low prevalences. *Salmonella* Newport comprised only 3.4% of the isolates recovered from individual samples and only 2.6% of the isolates recovered from composite fecal samples.

When comparing alternative sampling methods, such as composite fecal sampling, with what has been considered the “gold standard”—individual animal sampling—the results may be misleading. No differences in the sensitivity of pooled fecal samples compared with composite fecal samples were found when using individual culture results as the standard. Composite fecal sampling appeared to have lower specificity because it identified operations as culture positive that were classified as culture negative with the traditional methods. When the alternate method was used as the standard for comparison, the sensitivity of the traditional method was lower than expected. In this study, the sensitivity based on individual fecal samples was 71.9% and that based on pooled samples was 70.2% compared with composite fecal sample results.

In conclusion, composite fecal sampling provides results similar to those of individual and pooled fecal sampling methods in terms of the sensitivity of detecting *Salmonella* by culture and the identification of serovars. Results of composite samples did not differ by season, suggesting that these samples may be more cost effective than individual or pooled samples for determining a herd’s *Salmonella* status or identifying serovars present during the winter months. Composite fecal sampling is less costly and time-consuming than individual or pooled sampling for detecting the presence of *Salmonella* and may allow identification of more *Salmonella* serovars in dairy herds.

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