

Salmonella Prevalence Varies Over Time and Space in Three Large, Adjacent Cattle Operations in the Southwestern United States

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Hanson DL, Loneragan GH, Brown TR and Edrington TS (2022) Salmonella Prevalence Varies Over Time and Space in Three Large, Adjacent Cattle Operations in the Southwestern United States. Front. Anim. Sci. 3:878408. doi: 10.3389/fanim.2022.878408 We set out to describe the prevalence of Salmonella enterica in three large, adjacent cattle operations in the southern High Plains of the United States, Operations included two dairies (one of which routinely administers a commercially available Salmonella vaccine) and one feedlot. Samples were collected monthly for 12 months. At each sample collection, 25 freshly voided fecal pats and a sample from each of the water troughs were collected from each of five pens of cattle within an operation. Each monthly collection included a total of 375 fecal and \sim 32 water samples for a yearly total of 4,500 and 379 samples, respectively (note that the number of water troughs per pen varied within an operation). Salmonella was commonly recovered from fecal (71.3%) and water (28.5%) samples and tended to follow somewhat similar temporal patterns over time. However, its prevalence varied among operations despite being adjacent properties in that Salmonella was recovered from 61.3, 80.1, and 75% of fecal samples from dairy 1, dairy 2 and the feedlot, respectively. Salmonella prevalence in water samples across collection times averaged 36.1, 70.2, and 46.1% for dairy 1, dairy 2, and the feedlot, respectively. While it is uncertain why the Salmonella prevalence varied from operation to operation, the higher observed prevalence of Salmonella in water on dairy 2 and/or the use of a commercial Salmonella vaccine by dairy 1 may offer a partial explanation.

Keywords: Salmonella, feedlot cattle, dairy cattle, feces, water

INTRODUCTION

Salmonella continues to be a challenge for both livestock producers, meat processors, and public health officials. Cattle are natural reservoirs for *Salmonella* and while this pathogen can and does cause significant disease in some instances, most infections appear to be asymptomatic carriage (Fedorka-Cray et al., 1998; Edrington et al., 2004a) producing no readily detectible effects to the animal. *Salmonella* contamination of beef products for human consumption continues to occur despite significant improvements in beef processing including use of many successful post-harvest sanitation processing aids and interventions. Recently, a petition has arisen calling for the labeling of *Salmonella* as an adulterant as was done for *E. coli* O157:H7 and various other Shiga-toxin producing *E. coli* strains. In other work, *Salmonella* was readily recovered from peripheral,

non-mesenteric lymph nodes of cattle, where presumably it escapes many in-plant sanitary processes and unless physically removed by trimming, may be incorporated into trim used in the production to ground beef (Arthur et al., 2008; Li et al., 2015). Hence there is considerable interest in reducing the carriage of *Salmonella* in both beef and dairy cattle on the farm, to reduce the opportunity for disease, as well as improve the safety of our beef supply.

Considerable research has been conducted to better understand the ecology of *Salmonella* within a cattle operation (dairy, feedlot), as well as transmission dynamics of this pathogen among animals within an operation (Fitzgerald et al., 2003; Edrington et al., 2004a,b,c; Gragg et al., 2013; Brown et al., 2015). In general, *Salmonella* is more prevalent in cattle operations in the southern vs. northern latitudes of the United States, and more prevalent in the animals in the summer and early fall compared to the winter and spring (Gragg et al., 2013; Webb et al., 2017). Not surprisingly however, exceptions to these generally accepted paradigms occur. A fuller understanding of these exceptions and their determinants may provide important insight for the design and implementation of on-farm intervention strategies.

The southern high plains region of Texas and New Mexico is home to a significant proportion of the feedlots and dairies in the United States and it is not uncommon to find largescale operations in relatively close proximity to one another. In the current research, a longitudinal investigation was conducted on the fecal prevalence and concentrations of Salmonella on three adjacent cattle operations, two dairies and one beef feedlot. As water troughs have been implicated as a significant source of pathogen exposure (LeJeune et al., 2001), water samples were also obtained at each collection timepoint. Prior research had demonstrated that Salmonella is readily recoverable from cattle (both feedlot and lactating dairy cows) and their environments within this region of the United States. Given the unusual proximity of the enrolled operations described herein, we set out to describe within and among variation of Salmonella prevalence across operations, management practices, and cattle types.

MATERIALS AND METHODS

Two commercial dairy farms and one feedlot operation located in eastern New Mexico were enrolled in the study. These commercial cattle operations were adjacent to one another, and all located within a three km radius (**Figure 1**). The two dairies maintained ~2,200 and 6,500 lactating cows each, while the feedlot had a one-time capacity of 35,000 cattle. All operations were representative of dairy and feedlots within this region of the United States. One dairy used a commercially available *Salmonella* vaccine (*Salmonella* Newport Bacterial Extract vaccine with SRP[®] Technology, Zoetis Inc., Kalamazoo, MI, USA) according to manufacturer's recommendations (dairy 1) and the feedlot incorporated a direct-fed microbial (Bovamine[®], Chr Hansen, Hoersholm, Denmark) into the cattle diets for pathogen control. Dairy 2 did not use either of these products.

Sample Collections

Fecal and water samples were collected monthly for 1 year from each of the cattle operations. Twenty-five fecal samples were collected from each of five pens on each operation monthly (125 fecals/farm/month). On the dairies, pens housing animals nearest peak lactation (\sim 100 days in milk) were sampled, while on the feedlot, pens containing animals within 30 days of slaughter were collected. Freshly voided, undisturbed fecal pats were sampled utilizing clean plastic spoons (one per sample) to transfer the feces to individual specimen cups.

Water samples were collected from all water troughs in the pens in which fecal samples were obtained. Some pens shared water troughs such that with the exception of the first collection in which 32 water samples were collected, 27 water samples were collected monthly. A total of 4,500 fecal and 379 water samples were collected over the 12-month study period. All samples were placed in coolers on ice and shipped overnight to the USDA laboratory in College Station, TX for bacterial culture described below.

Bacterial Culture

Fecal and water samples were quantitatively and qualitatively cultured for Salmonella within 24 h of collection as described previously (Edrington et al., 2018). For quantitative culture, 10 g of each fecal sample was mixed with 90 ml of tetrathionate broth, one ml of this mixture removed and from that 50 µl direct plated on xylose lysine deoxycholate (XLD) agar (Oxoid, Basingstoke, UK) using a commercial spiral plater (Spiral Biotech Autoplate 4000; Advanced Instruments, Inc. Norwood, MA). The XLD plates were incubated (37°C for 24 h) and morphologically typical black colonies were counted and converted to log₁₀ CFU per gram of feces. For qualitative detection of Salmonella, the feces-tetrathionate mixture above was incubated overnight (37°C); after which, 100 µl of the enrichment was transferred to a second enrichment (5 ml Rappaport-Vassiliadis broth (RV; Remel Products, Lenexa, KS) and incubated at 42°C for 24 h. Following the second enrichment, each sample was streaked for isolation on brilliant green agar (Oxoid) containing novobiocin ($25 \mu g/ml$; BGA_{nov}) and incubated ($37^{\circ}C$, overnight). Presumptive Salmonella isolates were confirmed biochemically using lysine and triple sugar iron agars.

Water samples were vortexed and 1 ml of each sample removed and plated onto XLD agar using the spiral plater, incubated and counted as described above. For qualitative analysis, 25 ml of each water sample were enriched with the addition of 25 ml tryptic soy broth (TSB) and incubated overnight at 37°C. A secondary enrichment was conducted as above by transfer of 100 μ l of the TSB enrichment to 5 ml RV broth and incubated at 42°C for 24 h. Presumptive *Salmonella* isolates confirmed as above.

Statistical Analyses

Salmonella prevalence and concentrations were analyzed using quassibinomial and gaussian generalized linear models, respectively (R version 3.1.2, The R Foundation for Statistical Computing Platform). The quassibinomial model incorporated



dispersion parameters to account for either under- or overdispersion of data due to clustering within each cohort. Prevalence estimates were calculated for each operation individually by dividing the number of culture positive samples by the total number of samples collected monthly. Model-adjusted estimates of prevalence and imprecision were computed. For quantitative data, a missing value was recorded in instances where *Salmonella* concentration was below the limit of quantification and for those samples from which *Salmonella* was recovered (i.e., *Salmonella* was present at a concentration above the limit of detection) but below the limit of quantification (i.e., no morphologically typical colonies were observed using direct plating).

RESULTS

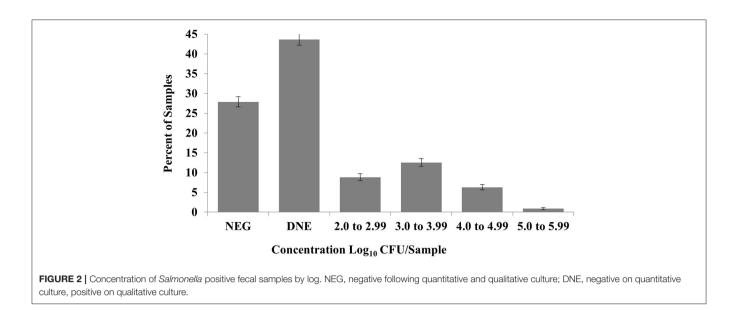
Of the 4,500 fecal and 379 water samples examined, *Salmonella* was recovered (following enrichment of samples) from 71.3% [n = 3,208; 95% confidence limit (CL) = 69.9, 72.6%] and 46.7% (n = 177; CL = 41.6, 51.7%) of fecal and water samples, respectively (**Table 1**). Of those with quantifiable concentrations of *Salmonella* (**Table 1**), the mean concentration of *Salmonella* in fecal samples was 3.27 log₁₀ CFU/g with a range of 2.08–5.68 log₁₀ CFU/g and 2.42 log₁₀ CFU/ml of water (range: 2.3–3.38 log₁₀ CFU/ml). For the fecal and water samples, 28.5 (n = 1,284; CL = 27.2, 29.8%) and 4.5% (n = 17; CL = 2.39, 6.6%) of the fecal and water samples contained concentrations above the

TABLE 1 | Prevalence and quantification of Salmonella in fecal and water samples collected from commercial dairy and feedlot operations within close proximity.

Sample	Concentration ^a	% Quantifiable	Prevalence, %	
Feces	3.27	28.5	71.3	
Range	2.08-5.68	2.08–5.68 8.3–47.5		
By operation				
Feedlot	2.19	19	75	
Dairy 1	2.57	16.5	61.3	
Dairy 2	3.46	50.1	80.1	
Water	Vater 2.42		46.7	
Range	2.3-3.38	0-21.9	3.1–93.8	
By operation				
Feedlot 2.19		6.1	46.1	
Dairy 1	2.57	2.8	36.1	
Dairy 2 2.2		5.9	70.2	

^aExpressed as log₁₀ CFU/g feces or log₁₀ CFU/ml water.

limit of quantification, respectively (**Table 1**). The distribution of concentrations in fecal samples is presented in **Figure 2** across operation and month of collection. The majority of samples were negative (by quantitative and qualitative culture) or negative following direct plating, but positive after enrichment. Of those samples quantitatively positive following direct plating, most samples fell in the range of 2.0–4.99 log₁₀ CFU/g feces, with only a small percentage of samples containing concentrations above $5.0 \log_{10}/g$ feces.



When examined across month of collection by operation, fecal prevalence estimates in enriched samples were 75 (n = 1,125; CL = 72.7, 77.2%), 61.3 (n = 919; CL = 58.7, 63.7%), and 80.1% (n = 1,202; CL = 78.0, 82.1%) for samples from the feedlot, dairy 1, and dairy 2, respectively (**Table 1**). The percentage of fecal samples with *Salmonella* concentrations above the limit of detection (% quantifiable) were 19 (n = 285; CL = 17.0, 20.9%), 16.5 (n = 248; CL = 14.6, 18.4%), and 50.1% (n = 751; CL = 47.5, 52.6%) for the feedlot, dairy 1, and dairy 2, respectively (**Table 1**). Fecal concentrations averaged 2.19, 2.57, and 3.46 log₁₀ CFU/g feces for the feedlot, dairy 1 and dairy 2, respectively (**Table 1**).

Salmonella prevalence estimates of enriched water samples (**Table 1**) were 46.1% (feedlot; n = 53/115; CL = 36.9, 55.2%), 36.1% (dairy 1; n = 65/180; CL = 29.1, 43.1%), and 70.2% (dairy 2; n = 59/84; CL = 60.4, 79.9%). The prevalence of *Salmonella* in water trough samples ranged from 3.1 (n = 1; CL = 0.1, 16.2%) to 93.8 % (n = 30; CL = 79.2, 99.2%) across all collections and operations. The percentage of water samples with quantifiable concentrations averaged 6.1 (n = 7; CL = 1.7, 10.4%), 2.8 (n = 5; CL = 0.3, 5.2%), and 5.9% (n = 5; CL = 0.9, 11.1%) for the feedlot, dairy 1, and dairy 2, respectively. Of these water samples, the mean concentration of *Salmonella* by operation was 2.19, 2.57, and 2.20 log₁₀ CFU/ml of water for the feedlot, dairy 1 and dairy 2, respectively (**Table 1**).

Fecal data is presented by month of collection and operation in **Table 2**. Variation in fecal *Salmonella* prevalence among the different operations was most pronounced from March through May and again in October through March, whereas the June through September prevalence was relatively consistent among operations and the greatest (74–100%). Dairy 2 had the greatest prevalence of *Salmonella* in fecal samples for 9 of the 12 collections. The percentage of samples with quantifiable populations showed considerable variation throughout the study, and like prevalence, was most frequently on dairy 2 (10 or 12 collections). Concentration of *Salmonella* in the feces in comparison was similar among operations in each of the collection months with one exception, April, in which dairy 2 did not have any fecal samples with quantifiable *Salmonella* concentrations. Otherwise, concentrations were relatively consistent month to month among operations, ranging from 2.67 to $4.06 \log_{10}$ CFU/g feces.

Due to the relatively few water samples with detectable *Salmonella* concentrations, *Salmonella* prevalence is presented in **Figure 3** by month of collection and operation. Not surprisingly, the summer months found the greatest prevalence of *Salmonella* in the water troughs. Dairy 2 demonstrated the greatest *Salmonella* water prevalence, with 100% of the water samples positive in June through November and over 50% incidence in February and March. Dairy 1 and the feedlot followed the same general month to month trends as dairy 2, with mostly lower prevalence at each collection.

DISCUSSION

To the best of our knowledge, this is the first descriptive study to explore Salmonella prevalence in different commercial cattle operations type (feedlot and two dairies) that are adjacent to one another, in that they share common property boundaries. Within animals, fecal prevalence when examined across month of collection, was relatively similar as were Salmonella concentrations, however, the likelihood of finding a sample with concentrations above the LOD was greater for dairy 2, as compared to dairy 1 and the feedlot. Salmonella prevalence within the water troughs, was likewise similar for the feedlot and dairy 1, but greater for dairy 2. The number of water samples with detectable concentrations was low across all operations as were the actual concentrations. The variation in Salmonella prevalence between the two dairies is somewhat surprising, as they are immediately adjacent each other, have similar pen and housing design, and utilize similar feedstuffs. Given the geographic co-location and similarity of housing and

TABLE 2 Comparison of prevalence, range, and pathogen load within and				
across operations by month of sample collection.				

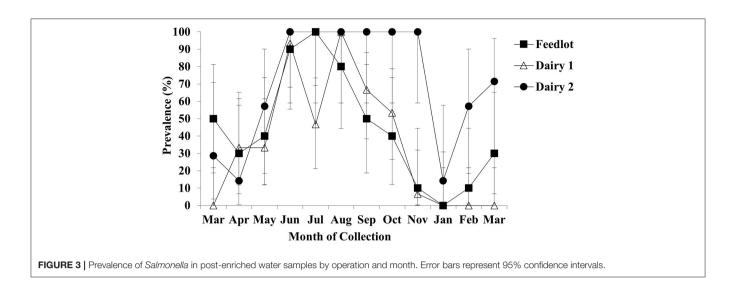
Month collected	Operation	Prevalence (%)	Range (%)	Quantifiable (%)	Concentration (log ₁₀ CFU/g)
	Feedlot	88	96–72	40	3.6
March	Dairy 1	21.6	32–12	5.6	3.04
	Dairy 2	9.6	16–0	1.6	2.89
	Feedlot	86.4	100–76	13.6	3.75
April	Dairy 1	80.8	96–48	11.2	3.28
	Dairy 2	38.4	56–20	0	0
	Feedlot	86.4	96–72	28	3.32
Мау	Dairy 1	63.2	72–52	21.6	3.4
	Dairy 2	82.4	96–64	34.4	3.74
	Feedlot	96.8	100–88	34.4	3.32
June	Dairy 1	95.2	100–88	41.6	3.11
	Dairy 2	98.4	100–92	69.6	3.36
	Feedlot	95.2	100–88	31.2	3.67
July	Dairy 1	84.8	92–76	17.6	2.93
	Dairy 2	100	100–88	93.6	3.93
	Feedlot	83.2	96–60	6.4	3.27
August	Dairy 1	97.6	100–92	41.6	3.24
	Dairy 2	100	100	87.2	3.5
	Feedlot	74.4	100–44	7.2	3.63
September	Dairy 1	89.6	96–76	28.8	3.02
	Dairy 2	100	100	72.8	3.5
	Feedlot	68	84–52	8	3.77
October	Dairy 1	76	88–52	7.2	2.67
	Dairy 2	99.2	100–92	63.2	3.38
	Feedlot	65.6	92–36	9.6	4.06
November	Dairy 1	60.8	72–48	18.4	3.65
	Dairy 2	97.6	100–88	72.8	3.84
	Feedlot	84.8	100–68	28.8	3.62
January	Dairy 1	48.8	72–28	1.6	2.7
	Dairy 2	85.6	92–72	33.6	3.12
	Feedlot	34.4	96–4	13.6	3.4
February	Dairy 1	8.8	16–4	2.4	3.22
	Dairy 2	87.2	96–80	49.6	3.49
	Feedlot	36.8	44–20	7.2	3.11
March	Dairy 1	8	16–4	0.8	2.99
	Dairy 2	63.2	96–20	22.4	3.39

cattle, other unmeasured factors such as general management, herd health programs and hygiene practices, could account for the variation in the prevalence of *Salmonella* across these operations. Breed could be a factor, as dairy 1 was a Holstein herd, whereas dairy 2 was Jerseys. To our knowledge there are no reports citing dairy breed accounting for *Salmonella* shedding differences. Another potential is the utilization of a commercially available *Salmonella* vaccine by dairy 1. Previous research with *Salmonella* vaccines and the effectiveness on *Salmonella* control in dairy cattle has yielded mixed results. In one study, fecal prevalence of *Salmonella* in animals culled from dairies utilizing a whole herd vaccination program was significantly lower than in animals culled from herds that were unvaccinated (Loneragan et al., 2012). Others reported no difference in fecal shedding of *Salmonella* in sub-clinically infected dairy cows (Heider et al., 2008; Hermesch et al., 2008) although in one study an increase in milk production was noted for vaccinated cows (Hermesch et al., 2008). Similarly, a vaccine effect was not observed in the prevalence of *Salmonella* in feedlot cattle (Dodd et al., 2011). Hence while the differences in prevalence between the two dairies could be attributed, at least in part, to the vaccine, it cannot be conclusively verified from this data.

There was a much greater prevalence of *Salmonella* in the water troughs on dairy 2 as compared to dairy 1 and the feedlot, which could explain the greater fecal incidence in that dairy. The frequency of water trough cleaning could partially explain these results as it was conducted every 2 weeks on the feedlot and dairy 1, compared to an as-needed basis for dairy 2. Chlorination of drinking water is deployed by some dairies in this region of the United States, but was not employed on any of the operations enrolled, to include the feedlot, in this study.

As cattle in feedlots are typically younger, fed different diets typically containing more concentrates, arrive from multiple sources and management practices, and are managed for very different outcomes than animals on dairies, fecal shedding of Salmonella as compared to the dairies was similar. The use of Bovamine was employed by the feedlot and not either dairy. While typically used for the mitigation of E. coli O157:H7, there is little evidence that Bovamine affects Salmonella to the same degree. That said, the current study design was not adequate to draw any conclusions around the use of Bovamine. In that sense, one ought to limit conclusions between the dairies and the feedlot included in this study to infer that the prevalence differed between these two production systems without generalizing more broadly to a wider population of feedlots and dairies. Obviously, a broader sample of enrolled, co-located feedlots and dairies would be needed for such inferential discussion. However, we are not aware of other situations where feedlots and dairies share such close (i.e., adjacent) proximity.

Further work may be warranted to explore and describe the unmeasured factors that account for operational-level variation in Salmonella prevalence. If such factors can be identified, an assessment of their practicality (i.e., their broad adoptability) and efficacy would be logical next steps (Wheeler et al., 2014). That said, this work highlights the significant variation that occurs in fecal shedding of cattle among operations within a small geographic region, as well as within an operation, hence research efforts may be better spent designing and evaluating interventions strategies than attempting to decipher the multitude of factors that may account for this variability. Regardless of management practices implemented on an operation, the environmental conditions that exist in this region of the United States, particularly wind, have the potential to affect pathogen dispersion both within and across operations. Such that efforts, such as vaccination, may have limited efficacy due to the infection pressure resulting from



the potential spread of *Salmonella* from one operation to the other. This could explain the elevated *Salmonella* incidence and the seemingly continual exposure to *Salmonella*, particularly in densely populated cattle producing regions such as examined in the current research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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ETHICS STATEMENT

Ethical review and approval was not required for the animal study because fecal samples were collected from pen floors with no distress to the animals.

AUTHOR CONTRIBUTIONS

DH and GL conceived the research and conducted sample collection. TE and TB cultured all the samples. All authors contributed to the article and approved the submitted version.

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