Epidemiol. Infect. (2008), **136**, 381–390. © 2007 Cambridge University Press
doi:10.1017/S0950268807008680 Printed in the United Kingdom

Investigation into the seasonal salmonellosis in lactating dairy cattle

T. S. EDRINGTON[1]*, T. T. ROSS[2], T. R. CALLAWAY[1], C. H. MARTINEZ[2], M. E. HUME[1], K. J. GENOVESE[1], T. L. POOLE[1], R. C. ANDERSON[1] AND D. J. NISBET[1]

[1] *USDA-ARS, Food and Feed Safety Research Unit, College Station, TX, USA*
[2] *Department Animal and Range Sciences, New Mexico State University, Las Cruces, NM, USA*

(*Accepted 11 April 2007; first published online 17 May 2007*)

SUMMARY

Sporadic salmonellosis has been reported in mature lactating dairy cattle in the southwestern United States and is an intriguing problem in that *Salmonella* can be cultured from faecal samples of these cattle throughout the year. However, it is pathogenic only during late summer/early autumn and in certain years. We sampled apparently healthy ($n=10$) and diarrhoeic ($n=10$) cattle during an outbreak on a 2000 head dairy in 2003. The following year, monthly faecal (from the same 30 head), total mixed ration, water, and pen soil samples were collected for *Salmonella* culture. No serogroup, serotype, genetic, or antimicrobial susceptibility differences were observed in comparison of isolates from healthy and sick cattle. During year 2 of the study, *Salmonella* was routinely cultured (although highly variable from month to month) from the cattle and the environment, although no outbreak of salmonellosis was observed.

INTRODUCTION

Dairy cattle serve as an important reservoir for *Salmonella* and have been implicated in cases of human salmonellosis [1, 2]. The United States National Animal Health Monitoring System's Dairy '96 study reported 5·4% of milk cows shed *Salmonella* and 27·5% of dairy operations had at least one cow shedding *Salmonella* [3]. *Salmonella* has been isolated from all ages of dairy cattle and throughout the production process. Mature dairy cattle typically appear asymptomatic while shedding this pathogen in their faeces [4–7] and while young calves are more susceptible to salmonellosis, cases in adult cattle have been reported [8–10].

Previous research conducted by our laboratory demonstrated significant variation in the prevalence of faecal *Salmonella* in healthy, lactating dairy cattle, not only among farms across the United States [11], but also in farms within a small geographic area and in individual farms from season to season [7]. Additional research examined production parameters (heifers *vs.* mature cows, lactation status, stage of lactation and heat stress) on *Salmonella* prevalence [6, 12]. While minor differences were noted in *Salmonella* shedding, results were generally inconsistent with no significant trends noted. Although heat stress did not result in any *Salmonella* shedding differences as measured by faecal incidence in the morning compared to the afternoon, in one experiment *Salmonella* prevalence averaged nearly 100% [6].

* Author for correspondence: Dr T. S. Edrington, USDA-ARS-FFSRU, 2881 F&B Road, College Station, TX 77845, USA (Email: edrington@ffsru.tamu.edu)
Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Outbreaks of salmonellosis in mature lactating cows have been reported on some of the farms we sampled previously, resulting in substantial financial losses due to decreased milk production and cow mortality (personal communication with producers). Interestingly, these outbreaks are seasonal, occurring in late summer/early autumn, but are not always an annual event. One farm in particular had an increased incidence of *Salmonella* shedding, even in the winter when the incidence of *Salmonella* typically decreases [7], compared to other regional dairies. In an attempt to elucidate factors involved in this seasonal salmonellosis, we examined *Salmonella* isolates obtained during an outbreak in 2003 from healthy and diarrhoeic cattle on this farm and then monitored 30 lactating cows, water, feed and pen soil samples on a monthly basis for 9 months the following year.

MATERIALS AND METHODS

2003 Salmonellosis outbreak

Faecal samples were collected during an outbreak of salmonellosis in the late summer of 2003 from mature lactating Holstein dairy cattle on a southwestern dairy farm (~2000 head). Cattle were maintained in large drylot pens, fed and managed as typical for dairy farms in this region of the United States. We were alerted to the outbreak in its final stages, therefore rectal grab samples could only be obtained from 10 diarrhoeic cattle that appeared to be in the early stages of salmonellosis. Ten apparently healthy lactating cattle from the same production group were also sampled at this time. We have sampled this dairy on multiple occasions [7, 12] and found *Salmonella* prevalence was often higher than neighbouring dairies and also quite variable from season to season in terms of overall prevalence, and serotype diversity. Based on our knowledge regarding *Salmonella* prevalence on this farm, we felt that sampling 10 healthy animals would provide for a reasonable assessment of the healthy cattle. If required, more samples would have been collected from healthy cattle the following week. All samples were cultured for *Salmonella* as described below.

2004 monthly surveillance

Mature multiparous Holstein dairy cattle on the farm described above were sampled on a monthly basis over a 9-month period (February to October 2004). Thirty head were selected that all calved within a 14-day period to eliminate potential animal differences associated with stage of lactation. Three cows were culled during the first 3 months of the experimental period from the sample population for various reasons, leaving 27 head remaining in the study group by October. Each monthly sampling was conducted in the morning with animals restrained in self-locking head stanchions. A palpation sleeve was utilized to obtain about 30 g faeces via rectal retrieval. Additionally, monthly samples of pen soil, water and the total mixed ration (TMR) were collected representative of the pens housing the study population of cows. Pen soil samples were collected from several locations within each pen using a soil probe which was disinfected between samples, from areas of full sun exposure and partial shade. Water samples were collected from each trough within a pen using sterile conical tubes. Multiple TMR samples (~100 g) were collected soon after feed presentation from the feed apron. Following each collection, all samples were placed on ice and shipped overnight to the USDA-ARS laboratory in College Station, TX for *Salmonella* culture and isolation described below.

Salmonella culture, isolation, serotyping and serogrouping

Salmonella was cultured by enriching about 10 g faeces in 90 ml tetrathionate broth (37 °C, 24 h), followed by a second enrichment in Rapport–Vassiliadis broth (100 μl in 5 ml, 42 °C, 24 h) prior to plating on Brilliant Green agar (BGA; Oxoid Ltd, Basingstoke, Hampshire, UK) supplemented with novobiocin (25 μg/ml). Following incubation (37 °C, 24 h), five colonies exhibiting typical *Salmonella* morphology were randomly selected from each sample and confirmed biochemically using lysine and triple sugar iron agars. Positive samples were re-streaked on tryptic soy agar with 5% sheep blood (Becton, Dickinson and Company, Sparks, MD, USA) for further confirmation and serogrouping, using slide agglutination with *Salmonella* O antiserum (Difco Laboratories, Detroit, MI, USA). *Salmonella* isolates were stored (−80 °C) using CryoCare™ bacterial preservers (Key Scientific Products, Round Rock, TX, USA). A portion of the isolates were sent to the National Veterinary Services Laboratory in Ames, IA for confirmatory serotyping. All media and agar were from Difco Laboratories (Detroit). Reagents and antibiotics were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Pulsed field gel electrophoresis (PFGE)

Select *Salmonella* isolates were analysed by PFGE as described previously [13]. Briefly, *Salmonella* isolates were thawed and spread on BGA plates and incubated as described above. A single colony from each plate was selected and incubated overnight in 10 ml TSB, centrifuged (8000 ***g***) and resuspended in 3 ml PBS. Washed cells were placed in a water bath (54 °C) and mixed with equal volumes of 1·8% (w/v) low melting temperature agarose in PBS. Cells with agarose were transferred to disposable plug moulds for polymerization (4 °C). Plugs were incubated overnight (50 °C) in 20 ml lysis buffer [2% (w/v) sodium lauryl sarcosine; 1·0 M EDTA (pH 9–9·3); and 0·2 mg/ml proteinase K] before washing twice (20 min, 4 °C) in TE [10 mM Tris (pH 8·0); 1 mM EDTA]. Plugs containing lysed cells were washed (3×; 20 min each) in 40 ml cold TE containing 40 μl phenylmethylsulfonyl fluoride (100 mM in isopropanol) and then washed three additional times (20 min) in cold TE. One half of each plug was incubated (20 min) with *Xba*I restriction endonuclease. Conditions for PFGE were: initial switch time = 0·1 s; final switch time = 90 s; included angle = 120°; 6 V/cm; buffer temperature = 12 °C; run time = 22 h. Genotypic relatedness was determined with Molecular Analysis Fingerprinting software, version 1.6 (Bio-Rad Laboratories, Hercules, CA, USA).

Antimicrobial susceptibility determination

Eighteen serotyped isolates each from the sick and healthy groups of cattle sampled in 2003 were examined for antimicrobial susceptibility using the Sensititre™ automated antimicrobial susceptibility system according to the manufacturer's directions (Trek Diagnostic Systems, Westlake, OH, USA). Broth microdilution was used according to methods described by the National Committee for Clinical Laboratory Standards (NCCLS) [14] to determine minimum inhibitory concentrations for the following antimicrobials: ampicillin, apramycin, ceftiofur, chlorotetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulphachloropyridazine, sulphadimethoxine, sulphathiazole, and trimethoprim/sulfamethoxazole. Resistance breakpoints were determined using the NCCLS interpretive standards [14] unless unavailable, in which case breakpoints in the United States National Antimicrobial Resistance Monitoring System (NARMS) 2000 Annual Report [15] were used. *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Enterococcus faecalis* ATCC 29212 were used as quality control organisms.

Table 1. *Comparison of* Salmonella *isolates cultured from faecal samples collected from healthy and diarrhoeic lactating dairy cattle* (*2003*)

	Healthy		Sick	
Item	*n*	%	*n*	%
No. animals sampled	10	—	10	—
No. *Salmonella* positive	10	100	10	100
Total no. *Salmonella* isolates	50	—	48	—
Serogroups and serotypes				
B	11	22	0	0
Agona	2	9·5	0	0
Banana	1	4·8	0	0
C_1	16	32	9	18·8
Livingstone	2	9·5	0	0
Mbandaka	0	0	1	3·8
Montevideo	3	14·3	4	15·4
C_2	0	0	9	18·8
Kentucky	0	0	5	19·2
E_4	2	4	10	20·8
Senftenberg	0	0	4	15·4
Taksony	1	4·8	0	0
G	6	12	7	14·6
Cubana	4	19	5	19·2
I	0	0	5	10·4
Barranquilla	0	0	2	7·7
K	15	30	5	10·4
Cerro	8	38·1	2	7·7
L	0	0	2	4·2
Minnesota	0	0	2	7·7
X	0	0	1	2·1
Bere	0	0	1	3·8
Total serotyped	21	—	26	—
No. different serotypes	7	—	9	—

RESULTS

A comparison of *Salmonella* isolates cultured from the faeces of diarrhoeic and healthy cows in 2003 is presented in Table 1. All 10 faecal samples from each group were positive for *Salmonella* and yielded 50 and 48 total isolates for the healthy and diarrhoeic cattle, respectively. Three of five serogroups (B, C_1 and K) accounted for 84% of the isolates in the healthy cattle compared to the diarrhoeic cattle in which six of eight serogroups (C_1, C_2, E_4, G, I, and K) represented 94%

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

of the isolates. Serogroup C_2 was not detected in any of the healthy *Salmonella* isolates while group B, which accounted for 22% of the healthy isolates, was not cultured in the faeceal samples of any of the sick cattle. Seven and nine different serotypes were identified in the healthy and sick isolates, respectively. The serotypes Senftenberg and Kentucky were not detected in any of the healthy cattle and accounted for 34% of the sick isolates. No differences in antimicrobial susceptibility patterns were observed in any the *Salmonella* isolates from sick and healthy cattle. Isolates were susceptible to all antimicrobials examined with the exception of spectinomycin, with three and five isolates resistant in the healthy and diarrhoeic groups, respectively (data not shown).

PFGE was used to compare the genetic relatedness of isolates cultured from the faecal samples of healthy and sick cattle. Seventeen serotypes representing 84 isolates were examined. No genotypic differences were noted when comparing sick *vs*. healthy isolates (Table 2). Multiple genotypes within serotype were observed for a number of the isolates examined.

Monthly *Salmonella* prevalence in faecal, soil, TMR and water samples collected in 2004 are presented in the Figure. Diarrhoea was not observed in any of the 30 cows during any of the monthly sample collections. Faecal prevalence ranged from a high of 96% in August to a low of 19% in October, averaging 54% over the 9-month period. Pen soil and TMR samples were also highly variable in *Salmonella* prevalence ranging from almost zero to 100% positive. The percentage of soil and TMR samples positive for *Salmonella* over the 9-month sampling period averaged 39% and 76%, respectively. A high percentage of TMR samples positive for *Salmonella* did not always correlate to a high percentage of positive faecal samples. Water sampled from the pen troughs also varied, ranging from 0% to 75% positive, averaging 38% positive across the 9-month experimental period.

Faecal *Salmonella* serogroup prevalence is presented by month in Table 3. Groups B and C_1 were most commonly identified, accounting for over 50% of the isolates each month except in August and October. In August, serogroup E_4 replaced B as the predominant group, while in October E_1, D_1 and K accounted for 80% of the isolates. The number of different serogroups identified in the faecal samples ranged from a low of four in May to a high of 11 in March and August. Serogroups of TMR isolates were predominantly C_1, E_1 and E_4 groups (Table 4).

Table 2. *Genotypes of selected* Salmonella *isolates cultured from healthy and diarrhoeic lactating dairy cattle* (*2003*)

Serotype	Genotype	No. isolates/ genotype	Animal status
Cubana	A	1	H
	B	1	H
	C	9	8 H, 1 S
Kentucky	A	7	4 H, 3 S
	B	2	2 S
Mbandaka	A	1	S
	B	1	H
	C	1	H
Soerenga	A	9	H
Senftenberg	A	5	4 H, 1 S
	B	4	H
Montevideo	A	2	H
	B	1	H
	C	11	H
	D	6	3 H, 3 S
	E	1	H
	F	1	S
Livingstone	A	1	H
Reading	A	1	H
Brandenberg	A	3	H
	B	1	H
Newport	A	2	H
Alachua	A	1	H
San Diego	A	1	H
Give	A	1	H
Oranienberg	A	1	H
Anatum	A	3	H
Minnesota	A	2	S
	B	1	H
Agona	A	1	H

Multiple serogroups (four or more) were identified in TMR samples every month with the exception of July. The number of serogroups identified from soil isolates was low in the first 6 months of the study compared to the final 3 months (August, September and October) averaging 2·2 and 7·7 different serogroups, respectively (Table 5). Groups C_1, B and K accounted for the majority of those identified from soil isolates over the 9-month period (Table 5). Similar to the other sample types, serogroup prevalence and diversity in water isolates varied by month over the course of the collection period, although in general fewer serogroups were identified (Table 6).

To examine serotype variation within an animal, we selected five cows that tested *Salmonella* positive (faeces) on the majority of the monthly samplings and randomly selected five isolates from each positive faecal sample for serogrouping. In previous research

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

Table 3. *Serogroup distribution* [*number and percentage of each month's isolates belonging to each serogroup* (*in parentheses*)] *of* Salmonella *isolates cultured monthly from faecal samples of healthy lactating dairy cattle over a 9-month period* (*2004*)

Serogroup	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
B	56 (21)	20 (25)	22 (34)	5 (18)	14 (25)	5 (12)	16 (13)	18 (26)	3 (12)
C_1	106 (39)	20 (25)	32 (49)	13 (46)	15 (27)	20 (48)	29 (24)	17 (24)	2 (8)
C_2	49 (18)	6 (7)	0	0	0	0	6 (5)	2 (3)	0
D_1	18 (7)	0	0	0	0	5 (12)	14 (11)	17 (24)	5 (20)
E_1	10 (4)	5 (6)	0	0	5 (9)	2 (5)	12 (10)	0	10 (40)
E_4	0	5 (6)	5 (8)	0	10 (18)	0	24 (20)	0	0
G	0	5 (6)	1 (1)	0	0	0	4 (3)	0	0
K	7 (3)	5 (6)	0	0	6 (11)	0	1 (1)	1 (1)	5 (20)
L	0	2 (2)	0	0	0	0	0	0	0
N	0	0	5 (8)	5 (18)	5 (9)	1 (2)	10 (8)	5 (7)	0
O	0	0	0	0	0	0	1 (1)	0	0
poly C	20 (7)	5 (6)	0	5 (18)	0	4 (10)	0	0	0
poly A-I, vi	3 (1)	7 (9)	0	0	0	5 (12)	5 (4)	10 (14)	0
Unknown	1 (0·3)	1 (1)	0	0	0	0	0	0	0
Total no. isolates	270	81	65	28	55	42	122	70	25
No. different serogroups	9	11	5	4	6	7	11	7	5

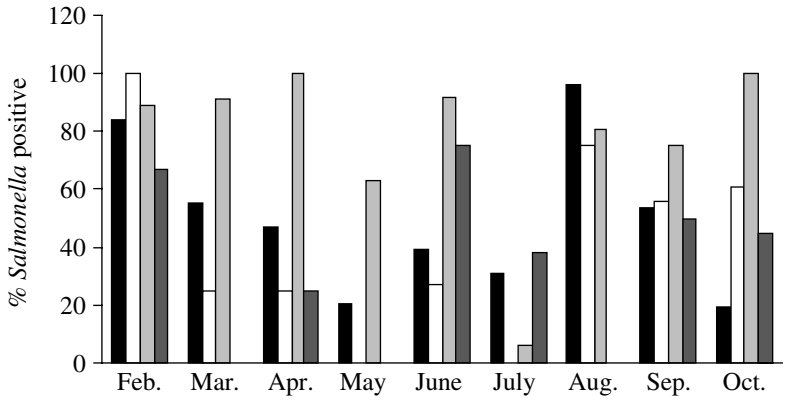

Fig. *Salmonella* prevalence (% positive) in faecal (■), soil (□), total mixed ration (▨), and water trough (▩) samples collected monthly over a 9-month surveillance period at a southwestern dairy.

conducted in our laboratory, *Salmonella* isolates cultured from the same faecal sample and belonging to the same serogroup, were often identified as the same serotype [7, 12]. Therefore in the current research, each isolate identified from an individual faecal sample and belonging to the same serogroup were assumed to be of the same serotype. Each isolate identified as belonging to a different serogroup were serotyped and the results presented in Table 6. This data highlights the serotype diversity and shifting prevalence within individual animals over time and demonstrates the difficulty in determining the optimum number of isolates required for serotype prevalence determination. One animal yielded four and another animal five different serotypes from one *Salmonella*-positive faecal sample (Table 7).

DISCUSSION

In an attempt to elucidate potential contributing factors to salmonellosis outbreaks in mature lactating dairy cattle, we examined isolates collected from diarrhoeic and healthy cattle during an outbreak in 2003. While the number of different serogroups and serotypes was greater in sick compared to healthy cattle, no differences between the two groups were particularly noteworthy with one exception. The serotypes Kentucky and Senftenberg were identified only

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

Table 4. *Serogroup distribution* [*number and percentage of each month's isolates belonging to each serogroup* (*in parentheses*)] *of* Salmonella *isolates cultured monthly from the total mixed ration* (*TMR*) *fed to healthy lactating dairy cattle over a 9-month period* (*2004*)

Serogroup	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
B	4 (11)	0	0	0	7 (12)	0	0	1 (1)	3 (5)
C_1	12 (34)	5 (11)	28 (70)	5 (25)	0	0	17 (26)	39 (49)	20 (34)
C_2	0	4 (9)	0	2 (10)	2 (3)	0	23 (35)	0	6 (10)
D_1	1 (3)	0	0	0	1 (2)	0	0	9 (11)	2 (3)
E_1	5 (14)	5 (11)	2 (5)	5 (25)	9 (15)	0	14 (22)	23 (29)	19 (33)
E_4	3 (9)	10 (23)	5 (13)	8 (40)	36 (61)	5 (100)	4 (6)	1 (1)	4 (7)
K	0	0	0	0	0	0	7 (11)	4 (5)	1 (2)
poly C	0	3 (7)	5 (13)	0	0	0	0	0	0
poly A-I, vi	10 (29)	10 (23)	0	0	1 (2)	0	0	1 (1)	3 (5)
Unknown	0	7 (16)	0	0	3 (5)	0	0	1 (1)	0
Total no. isolates	35	44	40	20	59	5	65	79	58
No. different serogroups	6	7	4	4	7	1	5	8	8

Table 5. *Serogroup distribution* [*number and percentage of each month's isolates belonging to each serogroup* (*in parentheses*)] *of* Salmonella *isolates cultured monthly from soil samples collected from pens housing healthy lactating dairy cattle over a 9-month period* (*2004*)

Item	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
B	4 (16)	0	2 (20)	0	2 (13)	0	5 (8)	3 (6)	10 (29)
C_1	16 (64)	10 (77)	0	0	0	0	14 (23)	13 (27)	2 (6)
C_2	1 (4)	0	0	0	0	0	14 (23)	4 (8)	0
D_1	0	0	0	0	0	0	7 (12)	5 (10)	5 (14)
E_1	0	3 (23)	2 (20)	0	0	0	5 (8)	1 (2)	4 (11)
E_4	0	0	5 (50)	0	8 (53)	0	0	0	0
G	0	0	0	0	0	0	5 (8)	4 (8)	0
K	0	0	0	0	0	0	1 (2)	10 (20)	14 (40)
N	0	0	0	0	0	0	0	3 (6)	0
poly A-I, vi	0	0	1 (10)	0	0	0	9 (15)	2 (4)	0
Unknown	4 (16)	0	0	0	5 (33)	0	0	4 (8)	0
Total no. isolates	25	13	10	0	15	0	60	49	35
No. different serogroups	4	2	4	0	3	0	8	10	5

in the sick cattle, with their respective serogroups accounting for a significant portion of the overall prevalence (19% and 21%, respectively) in comparison to the other serogroups identified. However, we have previously reported these serotypes in apparently healthy lactating dairy cattle [7, 12] and cannot attribute the salmonellosis observed in 2003 solely to these serotypes. Others have also reported isolating *Salmonella* Kentucky from healthy [3, 16–18] and diarrhoeic [9] dairy cattle. Serotype Barranquilla was also identified only in the sick group, however, only one cow had that particular serotype and serogroup. It seems likely that if Barranquilla isolates were the causative agent of illness, we would have identified that serogroup in other sick cattle.

Further analysis of the *Salmonella* isolates from sick and healthy cattle was conducted using PFGE. While multiple genotypes were identified within serotype, no distinguishable differences were observed when comparing sick *vs*. healthy isolates. Six different genotypes were observed within the Montevideo serotype out of 22 isolates examined. The Mbandaka and Cubana serotypes each contained three different genotypes while all nine isolates of the serotype

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

Table 6. *Serogroup distribution* [*number and percentage of each month's isolates belonging to each serogroup* (*in parentheses*)] *of* Salmonella *isolates cultured monthly from water samples collected from pens housing healthy lactating dairy cattle over a 9-month period* (*2004*)

Item	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
B	7 (23)	—	5 (50)	0	0	2 (13)	0	0	10 (40)
C_1	22 (73)	—	5 (50)	0	15 (100)	0	0	5 (25)	0
C_2	0	—	0	0	0	0	0	0	4 (16)
D_1	0	—	0	0	0	0	0	10 (50)	0
E_1	1 (3)	—	0	0	0	5 (31)	0	0	5 (20)
G	0	—	0	0	0	0	0	0	5 (20)
K	0	—	0	0	0	4 (25)	0	0	0
poly A-I, vi	0	—	0	0	0	2 (13)	0	5 (25)	1 (4)
Unknown	0	—	0	0	0	3 (19)	0	0	0
Total no. isolates	30	—	10	0	15	16	0	20	25
No. different serogroups	3	—	2	0	1	5	0	3	5

Soerenga were the same genotype. These results are similar to previous research reported by the authors in which multiple genotypes were observed for serotypes in which multiple isolates were examined including Montevideo, Mbandaka, and Cubana, however, as in this research the one exception was the serotype Soerenga, where only two genotypes were identified from 11 isolates [7].

We did not examine the faecal samples in 2003 for any pathogens other than *Salmonella* and relied on the attending veterinarian's diagnosis of salmonellosis being correct. Coupled with our examination that failed to identify any distinguishing differences among *Salmonella* isolates from sick and healthy cattle, it is possible that other pathogens may be responsible or contributing to the outbreak. However, similar outbreaks have occurred in these dairies previously and were diagnosed as salmonellosis. Unfortunately, at the time we did not quantify *Salmonella* concentrations in the faecal samples and in light of the current results, feel that this may have yielded more useful information that supports our hypothesis for the outbreak discussed below.

The following year we collected monthly samples from the same dairy that experienced the 2003 salmonellosis outbreak. Thirty cows all calving within 2 weeks of each other were sampled on a monthly basis over a 9-month period that incorporated the time-frame when salmonellosis outbreaks had previously occurred (August and early September). Water (from the trough), soil and TMR samples were collected, representative of the pens housing the cattle over the course of the collection period. Fortunately for the producer, the cows did not experience an outbreak of salmonellosis during 2004. The increase in overall faecal prevalence in June compared to May, and in August compared to July (particularly faecal prevalence), led us to believe that perhaps conditions were changing to favour an outbreak, but in September and October we observed a sharp decline in faecal prevalence as well as a shift in faecal serogroups.

Salmonella can survive for prolonged periods in the environment potentially contributing to chronic herd infection [8, 19, 20], therefore it was not surprising to isolate this organism from the pen soil and water samples. Somewhat unexpected, however, is the lack of a common serogroup between faecal samples and potential routes or infection or re-infection, namely feed, water and soil. In some months the same predominant serogroup was observed for all sample types, while in other months, serogroup varied with sample type. The diversity of serogroups observed in TMR samples is unexpected. *Salmonella* has been reported in animal and plant protein sources and by-product feeds commonly fed to dairy cattle [21–23] with a report that 40% of the protein animal by-product sources in the United States were contaminated with *Salmonella* [24]. However, while we attempted to collect samples that had not been disturbed by the cattle, we cannot rule out this as possible source of contamination. Similarly, insects, birds, and rodents are all possible contamination sources. Additionally, water was added to the TMR prior to

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

Table 7. Salmonella *serotype distribution by month for individual healthy dairy cows (2004). Five isolates per positive faecal sample serogrouped and only different serogroups sent for serotyping. Isolates identified as belonging to the same serogroup were assumed to be the same serotype*

Cow no.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
9722	Agona	Montevideo Senftenberg	Senftenberg	Neg.	Agona	Agona	Banana Senftenberg Alachua Montevideo Anatum	Give	Montevideo
2222	Anatum	Kentucky	Livingstone	Neg.	Neg.	Neg.	Kentucky Soerenga	Brandenburg Montevideo	Neg.
7354	Cerro Montevideo Brandenburg	Senftenberg Minnesota	Brandenburg Mbandaka	Neg.	Neg.	n.s.	Cerro Soerenga Senftenberg Kentucky	Neg.	Neg.
7424	Kentucky	Cerro	Soerenga	Soerenga	Soerenga	Soerenga	Soerenga	Soerenga	n.s.
8769	Montevideo Cerro	Cubana	Cubana	Montevideo	Anatum	Senftenberg	Montevideo	Fresno	Cerro

Neg., *Salmonella*-negative faecal sample; n.s., no sample.

feeding and may have served as a *Salmonella* source. In the second half of the experimental period, we collected water directly from the taps providing water to the troughs as well as the trough water. Only one tap sample was *Salmonella* positive which suggests that water may not have been the predisposing TMR contaminant source. Beginning on 23 March all drinking water was chlorinated on the farm as a means of *Salmonella* control. Due to the hardness of the water in this region, consistent chlorination was not achieved and the effectiveness of this control measure was difficult to determine. Possibly the chlorination was partially effective as only one tap-water sample was *Salmonella* positive, whereas continuous water trough contamination from cattle and other sources may have been sufficient to overwhelm the effectiveness of chlorine.

Results of the monthly collections highlight the extreme variation in *Salmonella* prevalence as well as serogroup and serotype diversity within a population of dairy cattle. We were not able to find any correlation of serogroup or serotypes in healthy or diarrhoeic cattle. Furthermore, we identified multiple serotypes from the same faecal sample on a number of occasions and have reported similar results previously [12]. In one animal, all five randomly selected isolates from a single *Salmonella*-positive faecal sample were identified as five distinct serotypes. Others have reported exactly the opposite. Wells and colleagues reported 12% of the dairies sampled had multiple serotypes, although culture-positive cows frequently had only one *Salmonella* serotype isolated [17]. In the San Joaquin Valley of California, *Salmonella* was cultured in 16% of the dairies, however, not more than one serotype was found on any one dairy [25]. Contrary to these reports and consistent with our research, the isolation of multiple *Salmonella* serovars was reported in a single California dairy [8]. Serotype diversity was reported to be greater for samples collected in the western United States compared to other locations [26, 27].

Asymptomatic *Salmonella* carriage and faecal shedding in adult dairy cattle is fairly common [6, 7, 16, 17], with salmonellosis typically associated with young calves. Clinical cases of salmonellosis have been reported in adult dairy cattle and the causative agent speculated [8–10]. Feed ingredients, diet changes, other infections and stress have all been suggested as potential contributors to salmonellosis in adult dairy cattle [10, 23, 28, 29]. We have sampled this dairy and others in the region on multiple occasions

https://doi.org/10.1017/S0950268807008680 Published online by Cambridge University Press

and frequently isolated *Salmonella* from faecal samples. As multiple serotypes have been isolated in these animals and based on the comparison of healthy *vs.* sick isolates, it is unlikely that a single serotype is responsible for the outbreak of salmonellosis. Over 2400 different *Salmonella* serotypes have been identified and all can be considered pathogenic depending on exposure level and host resistance [3]. A more plausible explanation may be achieved by examining the average monthly temperature data for the region. Ambient temperatures increase throughout the spring and summer, peaking at approximately the same time that previous salmonellosis outbreaks have been reported. Although measures to alleviate heat stress (e.g. shade, water misters) are employed by the farm, heat stress is an inherent part of dairy production in this region of the United States. We speculate that the salmonellosis outbreaks are a result of chronic heat stress that sufficiently weakens the animal's defence system to allow a rapid increase in overall *Salmonella* populations (not serotype specific) within the gastrointestinal tract thereby causing infection. Future outbreaks will include quantification of *Salmonella* populations throughout the gastrointestinal tract as well as culture for other pathogens that may be responsible in whole or part for the observed symptoms.

The research reported herein highlights the complexity of *Salmonella* control at the farm level. While reducing *Salmonella* prevalence on the farm has important cow health, environmental and food safety implications, this remains a daunting task. Previously we have attempted to identify potential factors contributing to *Salmonella* shedding that might pinpoint times or groups of cattle where implementation of *Salmonella* control measures would be most effective. Our research to date indicates that any on-farm control programmes will need to have broad reaching effects, encompassing animals at all ages and stages of production as well as the dairy environment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the excellent technical expertise of Kate Andrews and Reiley Street.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Center for Disease Control and Prevention.** *Salmonella* surveillance: Annual summary, 2001. Atlanta, GA: US Department of Health and Human Services, CDC, 2002.
2. **Zansky S, *et al.*** Outbreak of multidrug-resistant *Salmonella* Newport – United States, January–April 2002. *Morbidity and Mortality Weekly Report* 2002; **51**: 545–548.
3. **Wells S, *et al.*** *E. coli* O157:H7 and Salmonella – Status on U.S. Dairy Operations. USDA/APHIS/VS. Fort Collins, CO: Centers for Epidemiology and Animal Health, 1998.
4. **Richardson A.** Salmonellosis in cattle. *Veterinary Record* 1975; **96**: 329–331.
5. **McDonough PI.** Epidemiology of bovine salmonellosis. *Proceedings of the American Association of Bovine Practitioners Annual Meeting*, 1986; pp. 169–173.
6. **Edrington TS, *et al.*** Examination of heat stress and stage of lactation (early versus late) on fecal shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. *Foodborne Pathogens and Disease* 2004; **1**: 114–119.
7. **Edrington TS, *et al.*** Variation in the faecal shedding of *Salmonella* and *E. coli* O157:H7 in lactating dairy cattle and examination of *Salmonella* genotypes using pulsed-field gel electrophoresis. *Letters in Applied Microbiology* 2004; **38**: 366–372.
8. **Gay JM, Hunsaker ME.** Isolation of multiple *Salmonella* serovars from a dairy two years after a clinical salmonellosis outbreak. *Journal of the American Veterinary Medical Association* 1993; **203**: 1314–1320.
9. **Sato K, *et al.*** Spatial and temporal clustering of *Salmonella* serotypes isolated from adult diarrhetic dairy cattle in California. *Journal of Veterinary Diagnostic Investigation* 2001; **13**: 206–212.
10. **Anderson RJ, *et al.*** Case-control study of an outbreak of clinical disease attributable to *Salmonella* menhaden infection in eight dairy herds. *Journal of the American Veterinary Medical Association* 1997; **210**: 528–530.
11. **Callaway TR, *et al.*** Fecal prevalence and diversity of *Salmonella* species in lactating dairy cattle in four states. *Journal of Dairy Science* 2005; **88**: 3603–3608.
12. **Fitzgerald AC, *et al.*** Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. *Letters in Applied Microbiology* 2003; **37**: 392–398.
13. **Hume ME, *et al.*** Genotypic variation among Arcobacter isolates from a farrow-to-finish swine facility. *Journal of Food Protection* 2001; **64**: 645–651.
14. **National Committee for Clinical Laboratory Standards.** Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. National Committee for Clinical Laboratory Standards, 1999, Villanova, PA.
15. **Food and Drug Administration.** National Antimicrobial Resistance Monitoring System, Annual Report 2000 (http://www.cdc.gov/narms/annual/2000/tables/table_2.htm). Accessed 5 January 2002.

16. **Huston CL, *et al.*** Persistant fecal *Salmonella* shedding in five dairy herds. *Journal of the American Veterinary Medical Association* 2002; **220**: 650–655.
17. **Wells SJ, *et al.*** Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *Journal of Food Protection* 2001; **64**: 3–11.
18. **Animal Plant Health Inspection Service, Veterinary Services.** *Salmonella* on U.S. Dairy Operations: Prevalence and Antimicrobial Drug Susceptibility, 2005 (http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/dairy02/Dairy02_Salmonella.pdf.). Accessed 28 April 2006.
19. **Giles N, *et al.*** Persistence of *S.* Typhimurium in a large dairy herd. *Epidemiology and Infection* 1989; **103**: 235–241.
20. **Wray C, *et al.*** Epidemiology of *Salmonella* Typhimurium infection in calves: excretion of *S.* Typhimurium in the faeces of calves in different management systems. *Veterinary Record* 1987; **121**: 293–296.
21. **Franco DA.** The Animal Protein Producers Industry (APPI) *Salmonella* testing program. A profile of 1996 and the first two quarters 1997 results. *Proceedings of the Salmonella Committee, 1997 Annual Meeting*. United States Animal Health Association, Louisville, KY, pp. 24–21.
22. **McChesney DG, *et al.*** FDA survey determines *Salmonella* contamination. *Feedstuffs* 1995; **20**: 23.
23. **Wray C, Sojka WJ.** Reviews of the progress of dairy science: bovine salmonellosis. *Journal of Dairy Research* 1977; **44**: 383–425.
24. **Smith BP.** Salmonellosis. In: Smith BP, ed. *Large Animal Internal Medicine*. St. Louis: Mosby Year Book Inc., 1990, pp. 818–823.
25. **Pacer RE, *et al.*** Prevalence of *Salmonella* and multiple antimicrobial-resistant *Salmonella* in California dairies. *Journal of the American Veterinary Medical Association* 1989; **195**: 59–63.
26. **Galland JC, *et al.*** Diversity of *Salmonella* serotypes in cull (market) dairy cows at slaughter. *Journal of the American Veterinary Medical Association* 2001; **219**: 1216–1220.
27. **Troutt HF, *et al.*** Prevalence of *Salmonella* in cull (market) dairy cows at slaughter. *Journal of the American Veterinary Medical Association* 2001; **219**: 1212–1215.
28. **Moore GR, *et al.*** Bovine salmonellosis. *Journal of the American Veterinary Medical Association* 1962; **141**: 841–844.
29. **Van Dreumel AA, *et al.*** A common source epizootic of bovine salmonellosis in Manitoba. *Canadian Veterinary Journal* 1969; **10**: 33–44.