Experiences with *Salmonella* on California Dairies

JH Kirk, WM Sischo, SC Barnett, C Xiao, ACB Berge, DA Moore,
K Newman and ER Atwill
Veterinary Medicine Teaching and Research Center
School of Veterinary Medicine
University of California Davis
Tulare, CA

Salmonella infections in dairy calves and cows continue to be significant disease problems on California dairies. Calves continue to suffer from infectious diarrhea due to *Salmonella* as do adult dairy cows. As a result of these continuing problems, researchers at the Veterinary Medicine Teaching and Research Center lead by Bill Sischo have been studying *Salmonella* on dairies in the Central Valley of California, primarily in Tulare County. The purpose of this paper is to present some of the epidemiological findings of this research effort.

Characteristics of central California dairies

Tulare County has about 300 dairies with over 425,000 cows. The weather in the Central Valley varies from around 35 F to 110 F. The area usually receives rainfall from mid-November through the end of March with a yearly total of about 15 inches. More than half of the dairies use free-stall housing for their milking cows with seasonal access to dirt exercise lots. The most common bedding material is recycled manure from the lagoon separator that has been stacked for a period of time prior to using. Daily grooming and weekly replacement of the bedding is common. The remainder of the dairies use open corrals with manure and dirt. Most dry cows are kept in corrals. Corrals are groomed daily on some dairies; however, most do corral maintenance on a seasonal basis before and after the rains. The majority of corral dairies provide sunshades in each pen. Most dairies use a flush system to remove the manure from the housing and feeding areas. Alleys are flushed 2-6 times per day and it is not uncommon for cows to be in the alleys when they are flushed. The water used in the flush systems is usually recycled from the holding lagoons diluted with fresh water from the milking parlor sprinkler pens and misters at the feed bunks. Cows generally calve in covered, open corrals containing more than 5 cows, however, some dairies do have individual calving pens. Most calving areas have no additional bedding materials; however, some of the calving pens use straw, almond shells, cotton gin trash or rice hulls over dirt/manure. Fence line water troughs are generally shared between two groups of cows and common water troughs are usually located along the alleys leading to the parlor.

Birds

Our earliest efforts were to try to determine the extent of *Salmonella* carriage by wild birds commonly found on California dairies (1). None of the dairies were experiencing clinical salmonellosis in the cows or calves. In this study, birds were trapped or netted on 9 dairies (convinence sample of dairies). After the birds were caught, they were

euthanized and the entire GI tract collected for culture. Nearly 900 birds were included in the study and included 7 selected species (House Sparrow, House Finch, Starlings, Red-winged Blackbirds, Brown-headed Cowbird, Brewer's Blackbirds and Pigeons. About half the birds in the study were House Sparrows as they were very common on the dairies and easy to capture in the traps. *Salmonella* was isolated from 22 birds (22 of 892). The prevalence by dairy ranged from 0.7 to 16.7% while the species prevalence ranged from 1.2 to 3.2%. Cowbirds (3.2%) and House Sparrows (3.1%) had the highest prevalence. Five different serotypes were found in the House Sparrows. Five *Salmonella* serotypes were isolated (Meleagridis, Montevideo, Muenster, Typhimurium and an untypeable serotype). Over 40% of the *Salmonella* isolates were Meleagridis. There were only two Typhimurium and neither was multi-drug resistant.

Most of the *Salmonella* isolates were not those commonly isolated by the local veterinary diagnostic laboratory from sick animals in our area. They commonly find Typhimurium and it represents about 42% of the *Salmonella* isolates from sick animals. About 10% of the salmonella isolates from cattle are Montevideo. The bird isolates are also generally dissimilar to the distribution and types of *Salmonella* isolated from humans as reported in the FoodNet '99 results.

During the large *Salmonella* study to determine the extent of Typhimurium on dairies, we did experience a dramatic increase in isolations of multi-drug resistant, Newport on many dairies (2). On two of these dairies that had or were having clinical cases of salmonellosis in adult cattle, we attempted to determine the carriage rate in birds. There was a high rate of isolation of Newport in all environmental samples taken on both of these dairies (water, flush alleys, and feeds). From over 300 cow and environmental samples, 28% were positive for *Salmonella* and 34% of those were identified as Newport. Newport was only isolated from birds on one of the dairies. On that dairy, 14% of the captured House Sparrows were *Salmonella* positive. All were Newports.

From our findings, the overall prevalence of *Salmonella spp.* in birds is generally low (2 to 3%), however, some dairies may have much higher rates. There are seasonal changes in bird numbers with increases in transient birds to the dairies during the winter months. We suggest that the *Salmonella spp* carried by wild birds that frequent most California dairies are not a significant vector or reservoir of *Salmonella spp*. The most common bird on most dairies is the House Sparrow. These birds stay close the dairy where they were born and thus are probably not a significant threat to move salmonella to other nearby dairies. The exception to this generalization could be when large groups of starlings congregate on dairies. It is more likely that the *Salmonella* in the birds is only reflective of the *Salmonella* found elsewhere on the dairies from environmental locations.

<u>Water troughs for weaned calves</u>

In a study to determine the extent of *E. coli* O157 and *Salmonella* contamination of water provided the weaned dairy calves, we sampled water troughs on 48 dairies in the fall and repeated the attempts on 37 of the same dairies the following summer (3). Multiple samples were collected from each dairy from the waterers used by pen of calves that were

recently weaned off milk and moved out of individual housing. The dairies were scattered across California and selected by the local dairy livestock advisors as willing to cooperate in the study. In the fall, 4 of 82 samples were positive for *Salmonella* and in the summer 8 of 83 were positive. Only one dairy was positive in both seasons suggesting a lack of clustering by dairy. The method of filling the troughs and pH of the water were significantly associated with isolation of *Salmonella* from the water. Water in troughs filled by a continuous flow method were 5 times more likely to be contaminated with *Salmonella* than those with an on-demand valve (OR 5.6, 1.3,22.6, p=0.03). Water with pH>8 was 5.5 times more likely to have *Salmonella* compared to water with pH 6.2-7.9 (OR 5.5, 1.3,36.4, p=0.03). The was also a suggestion that water troughs could be cleaned too often. Though not significant, there was a tendency for troughs cleaned at intervals of less than 30 days (i.e. frequent cleaning) to be more likely to contain *Salmonella* than those cleaned at longer intervals. A tendency was also found for feeding antibiotics to the weaned calves. Water troughs in pens were calves were fed antibiotics tended to have higher risk of isolation of *Salmonella* compared to those troughs in pens where calves were not fed antibiotics.

Boots

Rubber boots were cultured for *Salmonella* after being worn in calving, hospital or fresh cow pens on 27 selected dairies (4). Dairies were selected for the study based on a willingness to participate, previous isolation of *Salmonella* in bulk tank milk or recent evidence of clinical *Salmonella* in adult dairy cows. On each dairy a new pair of boots was taken directly from the shipping box. In each case, boots were walked through the pens and when possible directly behind the cows when they were in the lockups. After about 25 paces, the boots were swabbed. Swabs were taken from the front, sides and bottoms of boots. Between 6 and 8 swabs were collected on each dairy. Some boots were also re-cultured 48 hours later after having been washed with the drop-hose in the milking parlor. No *Salmonella* was isolated from 15 of the 27 dairies. Five different *Salmonella* were isolated on the remaining 12 dairies. On seven dairies, two different *Salmonella* were isolated. The isolated *Salmonella* were Montevideo (10), Meleagridis (4), Newport (2), Typhimurium (1) and untyped (1). *Salmonella* was isolated on 1 of 4 boots that had been re-cultured after washing with drop-hose spray in the parlor on the dairy.

On the dairies where *Salmonella* had been previously found in the bulk tank milk, the same *Salmonella* were isolated on the boots. Additional *Salmonella* other than those found in the bulk tank milk were isolated from the boots. *Salmonella* Newport was isolated from both cows with clinical salmonellosis and boots on two dairies where clinical disease had recently been documented. These findings suggest that the *Salmonella* found on the boots was biologically related to the *Salmonella* associated with the cows and not just environmentally adapted serotypes. It also clearly demonstrates the need for proper washing and disinfection of boots when moving from place to place on a dairy. Simply spraying off the boots in the parlor as is commonly done on many dairies will not remove the *Salmonella* contaminates. In fact, it may only allow milkers' boots to become contaminated resulting in distribution of the *Salmonella* to the milking cow pens.

An alternate strategy would be to change boots before moving from a high risk area on the dairy to another area. It should also be kept in mind that it is not necessary to move an amount of *Salmonella* from one area to another area that is an infectious dose. The dairy environment is very conducive to bacterial growth. Indeed, in another study we demonstrated that bacteria could be carried at least 400 feet on the surface of boots (5).

Patterns of detection

In our larger study of salmonellosis on over 390 dairies from 2 milk cooperatives, the isolation of *Salmonella* from bulk tank milk was associated with herd size for some serogroups (6). The average herd size in this study was about 950 cows (range 64-6538). *Salmonella* in serogroup C1 showed the greatest increase in isolation as the herd size increased from less than 534 cows to greater than 1654 cows (OR 15.45) Other serogroups showed a similar tendency but to a much smaller extent (OR usually less than 5). For an unexplained reason, one cooperative was more likely to have dairies with *Salmonella* in the bulk tank than the other cooperative. *Salmonella* in serogroups B, C2 and E were more likely to be isolated during our cold months while serogroups C1 and D were more likely to be found during the cooler months. All serogroups were less likely to be isolated during our hot months of the summer. (See Table 2 for serogroups)

Distribution patterns on dairies

In the study of 390+ dairies in Fresno, Kings and Tulare counties in the central valley of California, about 50 dairies were heavily sampled to determine the extent of *Salmonella* in the cows and environmental sites. On each dairy, all the water troughs were sampled by taking a liter of water from the trough and by gathering biofilm from the trough sides and bottom. Fecal samples were taken from cows in each pen. About 20 samples were taken from cows in each pen. On average, cows per pen were between 100-150. Bedding samples were gathered from each pen. Each flush alley was sampled as at the outlet to the alley and at 2-3 location in the alley as the alley was being flushed. Samples were also taken from each of the feeds and commodities.

The initial purpose of the study was to compare the prevalence of S. Typhimurium on dairies, in local waterways and the local public health. During the study two unusual *Salmonella* serovars rose up within the study dairies. One of these, S. Newport, was not found at the beginning during several sampling period but was very prevalent during one of the last sampling periods. The other serovar, S. Reading, was particularly evident on a single dairy and found sparingly on 7 other dairies (Table 1). Nearly 80% of the isolates were found on one dairy.

Table 1 illustrates well the typical distribution of *Salmonella* isolates on the dairies that were studied. It is well to keep in mind that the distribution of samples over the various source sites was highest among fecal samples (20/pen) and much smaller among water sources (20-50/dairy) and flush system (<20/dairy). This distribution for S. Reading also shows that samples from the flush system and water will almost always allow isolation of most *Salmonella* serogroups and serovars present on any dairy.

Salmonella antibiotic resistance

In the larger study of *Salmonella* on 390 dairies, bulk tank milk samples were repeatedly taken from Sept 2000 through June 2002. Samples were collected at about 2 month intervals during 8 collection periods. Samples were taken by the tank truck drivers who delivered the milk to the 2 cooperatives. During these sampling periods, 40 to 70 bulk tank samples were positive for *Salmonella*. The *Salmonella* isolated included Montevideo, Dublin, Typhimurium, Give, Meleagridis, New Brunswick, Muenster, Cerro, Kentucky and Newport. In the laboratory, they were tested by disk diffusion for amikacin, amoxicillin, ampicillin, cephalosporin, cerftiofur, chloramphenicol, gentamicin, novabiocin, streptomycin, sulfas, tetracycline and trimethaprin sulfa. Only in the cases of Typhimurium and Newport was significant antibiotic resistance demonstrated. Over 70% of the isolates were sensitive to all the antibiotics in the screening panel (7).

Lagoon turnover rates

In the same large *Salmonella* study, nearly 50 dairies were visited for in depth sampling of cows and environmental sources of *Salmonella*. Of many sampling sites, lagoon water was studied (n=29). The rate of lagoon water turnover was estimated in the lagoons on the dairies by determining the amount of lagoon water recycled each day. The amount of lagoon water moved each day ranged from around 200,000 to nearly 3,000,000 gallons. The dairies were grouped by the length of time it would take to completely turn over the water in the lagoon. Those dairies with turn over rates of less than 30 days were more likely to have *Salmonella* isolated from the lagoon flush water than those with rates of greater than 30 days (8). Those dairies with mechanical separators in their manure management systems were found to be less likely to have *Salmonella* in the flush water than those dairies without mechanical separators (8).

References

1. Kirk JH, Holmberg CA, Jeffrey JS. Prevalence of *Salmonella spp* in selected birds captured on California dairies. JAVMA 220(3): 359-362, 2002.

2. Sakai MR, Kirk JH, Kinder D et al. Birds as vectors for *Salmonella enterica* Newport. Poster, CWARD, St. Louis, MO. 2002.

3. Kirk JH, Atwill ER, Holmberg C et al. Prevalence of and risk factors for *Salmonella* in water offered to weaned dairy calves in California, USA. Prev Vet Med 54:169-178, 2002.

4. Kirk JH, Sischo WM, Barnett SC et al. *Salmonella* contamination of rubber boots worn on dairies. The Bov Pract 36(1):11-14, 2002.

5. Kirk JH, Boggs C, Jeffrey J, Cardona C. Efficacy of disinfectants for sanitizing boots worn under dairy farm conditions. The Bov Pract 37(1): 50-53, 2003.

6. Barnett SC, Sischo WM, Moore DA, et al. Temporal patterns of *Salmonella* incidence on dairy farms. CWARD, St. Louis, MO. 2002.

7. Xiao C, Berge ACB, Barnett SC et al. Relationship between antibiotic resistance patterns of *E. coli* and *Salmonella spp*. isolated from bulk tank milk. CWARD, St. Louis, MO. 2002.

8. Newman K, Barnett SC, Xiao C et al. The effect of lagoon turnover rate on *Salmonella* in flush water systems. CWARD, St. Louis, MO. 2002.

Table 1. Distribution of S. Reading for various cow and environmental source sites on 8 dairies.

	Source of S. Reading isolates							
Farm	Bedding	Biofilm	Fecal	Flush Drain	Flush Valve	Water Trough	Water Well	Total
1							1	1
2			1			2		3
3		1		1		4		6
4		2	7	1		3	1	14
5					2	5	1	8
6						3		3
7	4	7	27	20	25	39		122
8	1							1
Total	5	10	35	22	27	56	3	158

Table 2. Common *Salmonella* serovars found on California dairies.

Serovar	B	C1	C2	C3	D1	E
	Tyhpimurium * Reading Saint Paul	Montevideo	Newport*	Kentucky	Dublin	Give Meleagridis Muenster New Brunswick

* Multi-drug resistant.