Prevalence of and risk factors for Salmonella in water offered to weaned dairy calves in California, USA

John Kirk[a*], Rob Atwill[a], Charles Holmberg[b], Marit Arana[c], Carol Collar[d], Dayna Ghirardelli[e], Gerry Higginbotham[f], Gary Markagaard[g], Denise Mullinax[h] ,Abraham Wubishet[i]

[a]Veterinary Medicine Extension, [b]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, 18830 Road 112, Tulare, CA, 93274; [c]University of California Cooperative Extension (UCCE) San Joaquin County, 420 S. Wilson Way, Stockton, CA, 95205; [d]UCCE Kings County, 680 N. Campus Dr., Hanford, CA, 93230; [e]UCCE Sonoma County, 2604 Ventura Ave, Santa Rosa, CA, 95403; [f]UCCE Fresno County, 1720 S Maple Ave., Fresno, CA, 93702; [g]UCCE Humboldt County, 5630 S. Broadway, Eureka, CA, 95503; [h]Hilmar Cheese Company, PO Box 910, Hilmar, CA, 95324, formerly UCCE Stanislaus County; [i]UCCE San Bernardino County, 777 Rialto Ave., San Bernardino, CA, 92415.

*Corresponding author. Tel.:559-688-1731; fax.:559-686-4231; email address:jkirk@vmtrc.ucdavis.edu

Abstract

Water from troughs used by weaned dairy calves was sampled on California, USA dairies to determine the extent of Salmonella contamination. Salmonella were found on 4 of 48 dairies (4/82 water samples) in fall 1998 and on 8 of the same 37 dairies (8/83 water samples) in summer, 1999. Serotypes isolated from the water were *Salmonella meleagridis* and *Salmonella typhimurium*. Primary risk factors associated with the increased presence of Salmonella in water offered to weaned dairy calves were a continuous (with overflow, versus valve) tank-filling method and water pH >8.0. Other secondary factors suggestive of increased risk were pattern of filling pens with new calves (continuously versus all-in, all-out), the cleaning of water trough at intervals ≤30 days (versus >30 days), absence of shade over water troughs and the presence of antibiotics in calf feed.

Keywords: water; calves; salmonella; risk factors

1. Introduction

The prevalence of salmonella on dairies in one county in California was 16% with a prevalence of antibiotic-resistant salmonella of 10.7% (Pacer et al., 1989). Another California study found that 84% of the dairies tested had at least one salmonella-positive cow in the herd (Smith et al., 1994). The NAHMS Dairy '96 study showed that 27.5% of 91 US dairy operations from 19 states had cows that were shedding salmonella (Wells et al., 1998). Salmonella are a common cause of human food-borne illness in the US (Wells et al., 1998); often, livestock are reported to be associated with these cases of human illness.

Factors associated with increased risk of Salmonella shedding were herd-size greater than 100 milking cows (OR=5.8, 95% CI 1.1, 31.3) (Kabagaambe et al., 2000); region of US with more in the South compared to the North (OR=5.7, 95% CI 1.4, 23.5) (Kabagaambe et al., 2000); free stall housing compared to other housing systems (Bender, 1994); other concurrent diseases such as Bovine Virus Diarrhea, Johne's Disease and fascioliasis (Bender, 1994); lack of on-farm quarantine practices versus having on-farm quarantine practices (Bender, 1994); vectors such as cats (OR=1.35, 95% CI 1.09, 1.65) (Evans, 1996); presence of rodents and birds (Bender, 1994) or evidence of wild bird access to feed stores (OR=1/67, 95% CI 1.11, 2.51)(Evans, 1996); purchase of replacements through dealers compared to purchase from other farms (OR=3.90, 95% CI 1.62, 9.36) (Evans, 1996); lack of dedicated sick pen compared to farms with dedicated sick pens (OR=1.51, 95% CI, 1.06, 2.16) (Evans, 1996); use of flush water systems (OR=3.5, 95% CI 0.9, 14.7) (Kabagaambe et al. 2000); using recycled lagoon water in flush system versus clean water flush systems (Gay et al., 1993); dietary stress due to marked changes in ration (House, 1998; Pelzer, 1989); using a particular feed mill (Anderson et al., 1997); feeding brewer's products (OR=3.4, 95% CI 0.9, 12.9) (Kabagaambe et al., 2000); feeding tallow (OR=2.3, 95% CI 1.0, 5.2) (Losinger et al., 1995); feeding whole cottonseed or cottonseed hulls (OR=3.5, 95% CI 1.0, 11.9) (Losinger et al., 1995) or animal fats (Anderson et al., 1997). Factors associated with reduced risk of Salmonella were using medicated milk replacers compared to not feeding medicated milk replacers (OR=.35, 95% CI .202, .606), feeding hay from 24 hours of age until weaning versus not feeding hay (OR=.52, 95% CI .311, .876) and calving in an

individual animal areas compared to other calving area systems (OR=.529, 95% CI .283, .987) (Losinger et al., 1995). Reported non-associated risk factors for Salmonella included, method of vermin control, access of animals to feeds, water sources (pond, lake or stream), use of calving area as a sick pen, use of water chlorination, frequency of water tank cleaning, floor moisture, frequency of manure application to pastures, feeding in open bunks and method of manure treatment (Kabagaambe et al., 2000).

Despite extensive studies, the prevalence of salmonella contamination of water offered to weaned dairy calves has not been reported. However, Salmonella might be found in water sources contaminated by infected animals and the water might be a potential source of infection for livestock (Pelzer, 1989). Our purpose of this observational, cross-sectional study was to determine the prevalence of Salmonella contamination in water offered to weaned calves and risk factors (environmental and management practices) associated with that prevalence.

2. Materials and Methods

University of California Cooperative Extension livestock advisors identified dairies in nine California USA counties for the study. No attempt was made to randomize the selection of the dairies; however, we did intentionally sample dairies in each of the major dairy areas of California. The counties ranged from southern to northern California (Table 1). The same dairies were visited in the fall 1998 (n=48) and summer 1999 (n=37) for collection of water samples and survey information. In the summer 1999, the dairies in Los Angeles and San Diego counties were not re-visited due to time and distance constraints. Three other central California dairies declined to participate in the summer sampling. At the time of the initial dairy visit (Fall 1998), owners or herdsmen were interviewed using a standardized questionnaire administered by the same investigator on all dairies for information about weaning, feeding and method of filling pens with calves (Tables 2 and 3). At the fall 1998 visit, water troughs were measured to the nearest inch while pens were measured to the nearest foot. Distances beyond 50 feet were estimated. Pen characteristics were observed on the initial visit. Filling methods for the troughs were observed at the Fall visit as classified as “continuous with valve” when a permanent attachment of the water source was made to the trough and the water level in the trough was controlled by a valve; “continuous with overflow” when a permanent attachment of the water source was made to the trough and water was continuously flowing to the trough without a valve to prevent overflow of water out of the trough; or “hose by hand, other” where the water source was not permanently attached to the trough as in filling with a hand-held hose.

All water troughs for calves weaned within the last 2 months were sampled on each dairy. The range in numbers of troughs sampled on the dairies was 1 to 4 troughs (Table 4). Due to expansion and re-modeling on some dairies, the calf pens and waterers used by the weaned calves had changed between the two sampling periods; in such instances, water troughs in different locations were sampled in the fall and summer. Water samples were collected in sterile, wide-mouth 500 ml jars by dipping through the water trough from the surface to the bottom. A consistent attempt was made to fill at least one-fourth of the jar with extraneous bottom materials, if present. Water samples were labeled and dated on the dairy and transported to the laboratory in an ice cooler surrounded by crushed ice. At the time of the dairy visit, the water pH and temperature

was measured for each trough sampled. The pH was measured with a Cole Palmer Waterproof pHTestr 2. Water temperature was measured within 6 inches of the water surface using a Weston Model 2292 thermometer. The chlorine content was measured in trough water where the owner reported using chlorination. A Leslie's Basic Poolcare OT Test Kit was used to test for chlorine content.

Extent of extraneous material (feed, straw, manure, algae) in the bottom of the trough was estimated qualitatively on a range from 1 to 3. A score of 1 indicated that less than 10% of the trough was filled with extraneous material. A score of 2 indicated that extraneous materials were present and filled $\geq$10% but <50% of the trough. A score of 3 indicated that $\geq$50% of the trough was filled with extraneous materials. On a "yes' or "no" basis, water was classified as "clear" if the bottom of the trough could be seen through the water in the trough. When extraneous materials were present at the bottom of the trough, a classification of "clear" indicated that the top of the extraneous material could be seen through the water.

One ml from the water sample for Salmonella isolation were inoculated into 10 mls of selenite enrichment broth and incubated at 37 C overnight (12-18 hours). Swabs from the selenite broth were streaked on selective plating media, Salmonella-Shigella (S-S) and further incubated at 37 C for 24-48 hours. Suspected Salmonella colonies (3-5) then were incubated on triple-sugar-iron (TSI) and urea media-slants and incubated for 24 hours at 37 C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen-sulfide production and negative for urea hydrolysis (yellow slant) were declared to be Salmonella-positive. Suspects were sero-grouped with commercial Salmonella sero-grouping reagent (Bacto-Salmonella Antisera, Difco Laboratories, Detroit, MI).

The association between each of the potential environmental and management risk factors and the odds of detecting Salmonella in the trough water sample was tested using logistic regression(Mehta and Patel, 1999). Forward stepping algorithm was used, with $P \leq 0.10$ for detecting whether the factor was significantly associated with the presence of Salmonella. The asymptotic likelihood-ratio test was performed for each factor to test the null hypothesis that the regression coefficients were simultaneously zero (odd ratio = 1) for the specified term. All survey factors (n=49) were submitted to analysis. Because we collected data on one to four troughs per dairy and the unit of interest was the individual trough, we tested the final fixed effects logistic regression model for dairy-level random effects using mixed-effects logistic regression with dairy as the random effect (Egret, 1997). Goodness-of-fit for the final multivariable model was calculated using both the deviance and the Hosmer-Lemeshow test, with a chi-square test performed on the appropriate degrees of freedom to determine P values (Mehta and Patel, 1999).

In order to test for multicollinearity between "selected" factors in the final model and factors not selected in the model, we used a simple Chi-square test with a *P*-value of 0.10 for evidence of potential multicollinearity. If we found that one or more of the "selected" model variables were associated with one or more of the "not-selected" variables, we then determined if the "not-selected" variables were significant in the final model following removal of the collinear "selected" variable, using a forward-stepping algorithm as described above.

Two of the 49 survey factors were categories for the statistical analysis. They were number of days between cleaning of the troughs and water pH. In essence, we split the number of observations in each of these survey factors near the mid-point.

3. Results

The average age of the weaned calves was between 110 –115 days in pens where the water troughs were sampled. Salmonella was isolated from the water troughs on 4 of 48 (9%) dairies and from 4 of 82 (5%) of the water samples during the fall 1998 and on 8 of 37 (22%) dairies and from 8 of 83 (10%) of the water samples during the summer 1999 sampling period. The serotypes isolated from the water were Salmonella meleagridis and Salmonella typhimurium. On only one of the 48 study dairies was Salmonella isolated during both sampling period. Salmonella meleagridis was isolated from the water during both sampling seasons. A single water trough out of 82 was positive for Salmonella on both samplings.

Based on the results of the fixed effects logistic regression model (Table 5), the odds of detecting Salmonella were over 5 times greater for water troughs continuously filled compared to troughs filled by a float valve. Adjusted for the method of filling, water troughs with basic pH conditions ($\geq$ 8.0) had 5-fold higher odds of Salmonella compared to troughs with more-acidic conditions (pH 6.2 to 7.9). We did not detect dairy-level random effects in our data (LRT on 1 df = 0.0, P = 0.5). Overall goodness-of-fit for the final fixed-effect model was: deviance = 1.1 on 1 df, P = 0.29; Hosmer-Lemeshow test = 0.14 on 1 df, P = 0.70.

Although we found several "not-selected" variables to be significantly associated with the "selected" final model variables (i.e., evidence for potential multicollinearity), none of these "not-selected" variables were significant as proxy variables in the final model (P-value >0.10). In other words, these "not-selected" variables could not function as proxy variables for those that were selected in the model.

Several other factors that had significant univariate associations ($P\leq$ 0.1; data not shown) were not included in the multivariate logistic regression model due to sparse data and despite utilizing exact methods (Table 6). Therefore, our concern is that we cannot properly assess the significance of these variables within our multivariate model (methods of filling and water pH). See suggestions…….

4. Discussion

Water was sampled in troughs used by weaned calf pens for two reasons. The original study for which the Salmonella portion was a secondary objective also focused on isolation of E. coli O157 from water. The literature indicated that for different ages of dairy cattle, just weaned dairy calves had the highest prevalence of fecal shedding for E. coli O157 (Hancock et al., 1997; Hancock et al., 1998; Gaber et al., 1999). In addition, in our immediate area in California, nearly 75% of the dairies send their pre-weaned calves off the home dairy at birth to be raised at calf ranches until they are weaned. For this reason, many dairies do not have pens of calves younger than 90-100 days of age available for sampling.

The isolation of *Salmonella typhimurium* in water from weaned dairy calves is an important finding. This particular Salmonella is well known for causing disease in both livestock and humans. On the other hand, finding *Salmonella meleagridis* is of less

interest because it is associated only rarely with disease in livestock or humans. Isolation of *Salmonella meleagridis* has been a common occurrence in another project involving culture of wild birds on California dairies (John Kirk, personal communication). The presence of Salmonella appears to be a random event without clustering as only a single, Salmonella-positive trough on one dairy was found.

Some risk factors associated with salmonella contamination of water provided to weaned dairy calves have not been previously identified either for water or for fecal shedding or outbreaks of clinical disease. Continuous filling of the trough, elevated pH and exposure to sunlight might provide an environment that is conducive to either growth or maintenance of salmonella, or perhaps might not allow competing microorganisms to utilize the growth niche preferred by salmonella. Perhaps valve filling of troughs may intermittently disturb the growth pattern for salmonella by aireation or stirring up competitors lodged in the slime of the trough thereby retarding salmonella growth.

Lack of off-farm quarantine has been reported to be a risk factor for increased clinical disease (Bender, 1994). We found in secondary analyses that continuously filling the pens with weaned calves increased the risk of salmonella contamination of the water troughs compared to all-in, all-out filling of the pens. Continuous filling of pens is similar to bringing animals directly onto the dairy: both allow mixing of stressed, "new" animals with other animals.

Frequent cleaning of water troughs is an oft-suggested "best-management practice" for dairies. However, a previous study by Kabagambe (2000) did not find an association between frequency of water trough cleaning and amount of fecal shedding of salmonella. Our study suggests, but does not define a causal relationship, that water troughs can be cleaned too often (in terms of salmonella contamination). Cleaning of water troughs at intervals $\leq$ 30 days perhaps disturbs a state of competitive exclusion present in the trough (either by opening up niches for salmonella or by removing bacterial competition). This effect may be similar to that of the valve-filling of troughs.

We found (secondary analyses) that feeding antibiotics to weaned calves was associated with increased prevalence of salmonella contaminated water troughs. A previous study by Losinger (1995) reported that feeding antibiotics to pre-weaned dairy calves reduced fecal shedding

Our findings suggest some practical management strategies to reduce the prevalence of Salmonella in the water. (However, keep in mind that the data supporting these suggestions are sparse and these are cross-sectional data.) For instance, to reduce the risk of Salmonella contamination in the water troughs, the data suggest:

1. Fill trough through a valve rather than continuously.
2. Keep water pH < 8.

Other steps to consider would be:

3. Use an all-in, all-out pattern of filling the pens.
4. Place shades over the troughs.
5. Clean regularly, but at intervals $\geq$ 30 days.
6. Reduce use of calf feeds containing antibiotics.

Many of the study factors that were not significantly associated with the presence of Salmonella in the water also have been examined in relationship to fecal shedding and

clinical disease. The use of flush alley-cleaning systems (Kabagambe et al., 2000) and recycled lagoon water through alleys (Gay et al., 1993) have been associated with fecal shedding. We found that flush alleys and their location relative to water troughs and recycled water were not associated with salmonella contamination of water troughs.

Factors non-associated with water contamination in our study were raising pre-weaned calves off the home dairy, group penning prior to weaning, average days at weaning, method of weaning, average age upon entering the weaned calf pens, duration of time in weaned pen, shape and construction material of water trough, dimension of the water trough, height above ground, water source, location of trough in pen, distance from adult cattle, distance from nearest feeding location, temperature of water, contamination of water with extraneous materials, chlorination of water, dimension of pen, sharing of waterers between pens, bedding used in pens, and calf density within pens.

While not directly studied in this project, reduction in exposure of calves to salmonella by reducing the extent of salmonella contamination of water offered to calves may decrease the fecal shedding and clinical disease caused by salmonella in dairy calves. These calves will also be exposed to fewer bacteria that have a potential to be a human food borne pathogen. In the long term, a safer food product will be marketed from the dairy.

5. Conclusions

To reduce the risk of Salmonella contamination of water being offered to weaned dairy calves, trough should be filled through a valve and not be continuous flow and water pH should be kept < 8. Other secondary factors to consider would be an all-in, all-out pattern of filling the pens with calves instead of continuously adding and removing calves; troughs should be cleaned regularly, but at intervals of perhaps 30 days or longer; shades should be placed over the troughs to prevent direct exposure to sunlight and feeds should not contain antibiotics.

References

Anderson, R.J., Walker, R.L, Hird, D.W., Blanchard, P.C., 1997. Case-control study of an outbreak of clinical disease attributable to *Salmonella menhaden* infection in eight dairy herds. J. Am. Vet. Med. Assn. 210(3), 528-530.

Bender, J., 1994. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. The Bov. Pract. 28, 62-65.

EGRET Reference Manual, 1997. Revision 4. Cambridge, MA: Software Corporation.

Evans, S.J., 1996. A case control study of multiple resistant Salmonella typhimurium DT 104 infection of cattle in Great Britain. Cattle Practice 4(3), 259-263.

Garber, L., Wells, S., Schroeder-Tucker, L., Ferris, K., 1999. Factors Associated with Fecal Shedding of Verotoxin-Producing Escherichia coli O157 on Dairy Farms. J Food Protection 62(4), 307-312.

Gay, J.M., Hunsaker, M.E., 1993. Isolation of multiple Salmonella serovars from a diary two years after a clinical salmonellosis outbreak. J. Am. Vet. Med. Assn. 203(9), 1314-1320.

Hancock, D.D., Besser, T.E., Rice, D.H., Herriott, D.E., Tarr, P.I., 1997. A longitudinal study of Escherichia coli O157 in fourteen cattle herds. Epid. Infection 118, 193-195.

Hancock, D.D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriott, D.E. and Carpenter, L., 1998. Multiple sources of Escherichia coli O157 in feedlots and dairy farms in Northwestern USA. Prev. Vet. Med. 35, 11-19.

House, J.K, Smith, B.P., 1998. Current strategies for managing salmonella infections in cattle. Vet. Med. August, 756-764.

Kabagambe, E.K., Wells, S.J., Garber, L.P., Salman, M.D., Wagner, B., Fedorka-Cray, P.J., 2000. Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. Prev. Vet. Med. 43, 177-194.

Losinger, W.C., Wells, S.J., Garber, L.P., Hurd, H.S., 1995. Management factors related to *Salmonella* shedding by dairy heifers. J. Dairy Sci. 78, 2464-2472.

Losinger, W.C., Garber, L.P., Smith, M.A., Hurd, H.S., Biehl, L.G., Fedorka-Cray, P.J., Thomas, L.A., Ferris, K., 1997. Management and nutritional factors associated with the detection of Salmonella sp. from cattle fecal specimens from feedlot operations in the United States. Prev. Vet. Med. 31, 231-244.

Mehta, C. and Patel, N., 1999. LogXact 4 for windows: users manual. Cytel Software Corporation, Cambridge, Massachusetts.

Pacer, R.E., Spika, J.S., Thurmond, M.C., Hargrett-Bean, N., Potter, M.E., 1989. Prevalence of Salmonella and multiple antimicrobial-resistant Salmonella in California dairies. J. Am. Vet. Med. Assn., 195(1), 59-63.

Pelzer, K.D., 1989. Salmonellosis. J. Am. Vet. Med. Assn. 195(4), 456-463.

Smith, B.P., Roden, L.D., Thurmond, M.C., Dilling, G.W., Konrad, H., Pelton, J.A., Picanso, J.P., 1994. Prevalence of salmonellae in cattle and in the environment on California dairies. J. Am. Vet. Med. Assn. 205(3), 467-471.

Wells, S.J., Ott, S.L., Seitzinger, A.H., 1998. Key health issues for dairy cattle – New and old. J. Dairy Sci. 81, 3029-3035.

Table 1. Location by California, USA county and number of dairies that participated in the housing and water trough sampling survey during 1998-1999

County	Location	Fall Sampling (n=48)	Summer Sampling (n=37)[a]
Fresno	Central	5	5
Humboldt	North	4	4
Kings	Central	5	5
Los Angeles	South	5	0
San Diego	South	3	0
San Joaquin	Central	5	5
Sonoma	North	5	5
Stanislaus	Central	6	4
Tulare	Central	10	9

[a] Eleven dairies that participated in the Fall did not participate in the summer, otherwise, all the dairies sampled in the Fall were sampled in the Summer.

Table 2. Weaned-calf management practices found on 48 California, USA dairies. "*" indicates a factor significantly associated with the isolation of Salmonella from a water trough sample.

Risk factor	Salmonella isolated from number of water troughs	
	Yes	No
Pre-weaned-calf management		
Pre-weaned calves raised off dairy		
Yes	3	44
No	8	97
Weaning method		
Abrupt	4	63
Gradual	4	43
Group penned prior to weaning		
Yes	5	63
No	4	58
Fed grain starter		
Yes	6	103
No		1
Fed hay		
Yes	4	49
No	2	55
Fed water		
Yes	4	79
No	2	17
Fed milk		
Waste milk	4	21
Whole non-waste milk	0	20
Milk replacer	1	14
Whole + replacer	1	12
Waste milk + replacer		22
Waste + whole + replacer		11
Wean-calf management		
Method of filling pen with calves		
Continuous	11	99
All-in; all-out	1	49
Shared water troughs between pens		
Yes	8	78
No	4	75
Number of troughs per pen		
One	12	132
Two	0	21
Shade covers over calves		
Yes	6	89
Partial	4	16
No	2	45

Flush system in calf pen		
Yes	5	35
No	7	109
Fed grain mix		
Yes	6	107
No	4	25
Fed silage		
Corn	1	10
Hay	6	36
No	2	56
Both		10
Fed commodity feeds		
Yes	2	16
No	7	62
Antibiotic fed in feed		
Antibiotic	4	14
Deccox	5	81
Other		4
None		15
Antibiotic + Deccox		4

<u>Water trough characteristics</u>

Shape		
Square	0	
Rectangle	12	
Round	0	
Trough construction material		
Acrylic covered	3	30
Concrete	11	89
Plastic	0	7
Metal	0	27
Method to fill trough		
Continuous with valve	8	135
Continuous with overflow	4	9
Hose or other	0	2
Water trough under shade		
Yes	1	45
No	11	97
Concrete apron under trough		
Yes	8	75
No	3	68
Partial	1	3
Water source		
City water supply	0	4
Dairy well	12	146
Other	0	3

Water chlorinated		
Yes	2	4
No	10	149

Table 3. Weaned-calf management practices found on 48 California dairies. "*" indicates a factor significantly associated with the isolation of Salmonella from a water trough sample.

Risk factor	Salmonella isolated from number of water troughs					
	Yes			No		
	Min	Med	Max	Min	Med	Max
Pre-weaned-calf management						
Number calves per pen	16	20.5	50	5	20	50
Age at weaning	45	90	108	45	90	120
Days off dairy	0	0	100	0	0	120
Weaned-calf management						
Number of calves per pen	14	32.5	50	4	25	94
Age enter calf pens	42	70	150	42	90	150
Age of calves in pens	60	90	210	60	120	210
Days stayed in pens	30	60	180	14	60	300
Calf pen characteristics						
Length, feet	60	100	300	25	100	500
Width, feet	25	75	100	15	75	250
Area, $feet^2$	1800	10000	22500	450	6000	62500
Area per calf, $feet^2$	46.9	363.3	666.7	44.4	281.3	2717.4
Water trough characteristics						
Diameter, inches	0	0	0	24	36	36
Length,inches	48	72	144	12	60	180
Width, inches	12	18	24	24	36	36
Depth, inches	6	18	24	6	18	36
Volumn, $inches^3$	3456	23328	46656	864	23338	352836
Surface area, $inches^2$	576	1296	2592	144	1296	9801
Surface above ground,inches	24	33	36	12	30	36
Nearest feeder, feet	2	13.5	20	0	12	75
Nearest cows, feet	30	175	500	0	200	1000
Nearest flush alley, feet	0	0.5	2000	0	0	2000
Days between cleaning	7	30	365	0	100	365
Water characteristics						
Trough water pH	7.5	8.55	8.7	6.2	7.8	10
Trough water temperature	50	71	99	36	65	88
Chlorine, ppm	0	0	3	0	0	3

Table 4 - Number of water samples collected on each California, USA dairy by sampling period.

Samples per dairy	Fall 1998 Dairies	Summer 1999 Dairies
1	19	2
2	24	26
3	5	7
4	0	2
Total	48	37

Table 5 – Final logistic regression model for factors associated with *Salmonella* in water troughs on commercial dairies in California.

Factor	Adjusted odds ratio	(90%Exact C.I)	Likelihood ratio test
How is the water trough filled			0.03*
Valve	1.0**	-	
Continuous	5.6	(1.3, 22.6)	
Water trough pH			0.03
6.2 to 7.9	1.0**		
>8.0	5.5	(1.3, 36.4)	

*Tests the null hypothesis that all logistic regression coefficients are simultaneously zero (odd ratios=1) for the specific terms, using the exact conditional score test.

**Referent category for the odds of *Salmonella* contamination.

Overall goodness of fit: deviance= 1.1 on 1 df, P-value=0.29; Hosmer-Lemeshow test=0.14 on 1 df, P-value=0.70.

Table 6—Factors associated with *Salmonella* contamination of water troughs on commercial dairies in California, USA.

Factor	Prevalence of *Salmonella*		Crude odds ratio	(90% C.I.)	Likelihood ratio test
Number of days between cleaning the trough					0.04†
>30	5/114	(3.9%)	1.0*		
0 – 30	7/51	(13.7%)	3.7	(1.3, 9.5)	
How is the water trough filled					0.009
Valve	8/143	(5.6%)	1.0*	-	
Continuous	4/13	(30.8%)	7.5	(2.7, 23.8)	
Is the trough covered by a shade structure					0.06
Yes	1/46	(2.2%)	1.0*	-	
No	11/108	(10.2%)	5.1	(0.9, 29.2)	
Water trough pH					0.006
6.2 to 7.9	2/88	(2.2%)	1.0*	-	
>8.0	10/77	(13.0%)	6.4	(1.7, 23.6)	
How do you fill your weaned calf pens					0.05
All in / all out	1/50	(2.0%)	1.0*	-	
Continuously	11/110	(10.0%)	5.4	(1.0, 31.1)	
Type of antibiotic added to feed					0.05
Antibiotic	4/18	(22.2%)	1.0*	-	
Deccox	5/87	(5.8%)	0.2	(0.05, 1.0)	
Antibio + Deccox	0/14	(0.0%)	0.2	(0.0, 1.3)	
Other	0/4	(0.0%)	0.8	(0.0, 5.5)	
None	0/15	(0.0%)	0.2	(0.0, 1.2)	

† Tests the null hypothesis that all logistic regression coefficients are simultaneously zero (odds ratios=1) for the specified term, using asymptotic likelihood ratio test.
* Referent category for the odds of *Salmonella* contamination.