Final Report

Project Title:

Methods to Control *E. coli* O157:H7 in Drinking Water for Cattle

Principal Investigator:

Michael P. Doyle

Institution:

Center for Food Safety
University of Georgia
Griffin, GA 30223

Stated Objective:

Our objective was to develop a practical method to control *E. coli* O157:H7 in drinking water for cattle and reduce the likelihood of transmission of *E. coli* O157:H7 through drinking water in the trough on the farm.

Summary of the project

Drinking water for cattle is an important vehicle of *E. coli* O157:H7 transmission. Survival of *E. coli* O157:H7 in water contaminated with rumen content at different water:rumen content or water:feces ratios, *E. coli* O157:H7 cell numbers, and temperatures was determined. At 21°C, *E. coli* O157:H7 inoculated at a high inoculum ($10^{5.8}$ cfu/ml) survived for 8, 15, 23, >56 and 24 weeks and at a low inoculum ($10^{2.9}$ cfu/ml) survived for 8, 11, 10, 11 and 10 weeks at a water:rumen content ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively. Different treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate and competitive inhibition *E. coli* were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm) and ozone treatment (22-24 ppm at 5°C or 8-12 ppm at 21°C) of water had a minimal effect on killing *E. coli* O157:H7 in the presence of rumen content at ratios of 50:1 and higher. Treatment by competitive inhibition *E. coli* in water with rumen content had minimal effect on *E. coli* O157:H7 counts compared with untreated controls. Four chemical treatment combinations including: (a) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid (Treatment A); b: 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate (Treatment B); (c) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid (Treatment C); (d) 0.1% lactic acid, 0.9% acidic calcium sulfate and 100 ppm chlorine dioxide (Treatment D) were

highly effective at 21°C in killing *E. coli* O157:H7, O26:H11 and O111:NM/ml in water heavily contaminated with rumen content (ratio of 10:1 water:rumen content, v/w) or feces (ratio of 20:1, water:feces, v/w). Among them, Treatments A, B and C killed >5 $\log_{10}$ *E. coli* O157:H7, O26:H11 and O111:NM/ml within 30 min in water containing rumen content. For Treatment D, *E. coli* O157:H7, O26:H11, and O111:NM were reduced within 30 min by 2.8, 4.3, and 3.2 log cfu/ml in water containing rumen content, respectively, and by 3.5, 4.9, and 4.6 log cfu/ml in water with feces, respectively. Cattle fed ad libitum water containing Treatment A, C, or Control for two treatment periods at 7-day increments drank 15.2, 13.8, and 30.3 L/day, respectively. Cattle fed water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 L/day. The amount of water consumed for all water treatments was significantly different from the control, and there were no significant differences among water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow body weight (BW) was converted to MBW ($BW^{0.75}$), and intake of water per MBW was calculated. Treatment effects for water intake/MBW were similar to those for total water intake.

Background Information

E. coli O157:H7 has emerged in the last 10 years as an important foodborne pathogen; with an estimated 73,000 cases annually in the U.S. Cattle are the major reservoir and studies revealed that when present in cattle drinking water, *E. coli* O157:H7 was disseminated to other cattle using the contaminated water source.

Genomic subtyping of *E. coli* O157:H7 isolates from farms by pulsed-field gel electrophoresis has revealed that a single O157:H7 strain is dominant among isolates from cohort and noncohort cattle, water, and other positive samples (i.e., from feed, flies, and a pigeon, etc.) on a farm. This indicates that drinking water is an important vehicle for disseminating *E. coli* O157:H7 on the farm.

Studies indicate that once contaminated in the drinking water of the cattle farm, *E. coli* O157:H7 it will survive for a long period of time. A variety of treatments have been evaluated for their efficacy in killing *E. coli* O157:H7 in drinking water contaminated with cattle feces. Results revealed that most had minimal effect on killing *E. coli* O157:H7 because these treatments were neutralized by organic materials present in the rumen content or feces. The objective of this study was to identify practical treatments to eliminate or control *E. coli* O157:H7 in drinking water simulating on farm conditions.

Materials and Methods

Bacterial strains: Five isolates of *E. coli* O157:H7, including 932 (human isolate), E009 (beef isolate), E0018 (cattle isolate), E0122 (cattle isolate), and E0139 (deer jerky isolate); five isolates of *E. coli* O26:H11, including strains DEC10E (cattle isolate), DEC9E (cattle isolate), DEC10B (cattle isolate), 3079-97 (human isolate), and 3183-96 (human isolate); and five strains of *E. coli* O111:NM, including strains 3208-95 (human isolate), 0944-95 (cattle isolate), 3287-97 (human isolate), 4543-95 (cattle isolate), and 0073-92 (cattle isolate) were used in this study. To facilitate the enumeration of these bacterial isolates, all strains of *E. coli* O26:H11, O111:NM, and O157:H7 were selected for resistance to nalidixic acid (50 µg/ml) according to the procedures reported previously (Brown, et al. 1997, and Zhao, et al. 1998). Each strain was grown individually in 10 ml of tryptic soy broth (TSB, Becton Dickinson Microbiology Systems, Sparks, MD) containing 50 µg of nalidixic acid (Sigma Chemical Co., St. Louis, MO) per ml for 16-18 h at 37°C with agitation (150 rpm). The bacterial cells were sedimented and washed three times in 0.1 M phosphate-buffered saline, pH 7.2 (PBS) by centrifugation (4,000 X g, 20 min), and resuspended in PBS. Cells were adjusted with PBS to an optical density at 640 nm of 0.5 (approximately 10^8 CFU/ml). Five strains were combined at equal concentrations. The populations of each individual strain and the five-strain mixture were confirmed by enumeration on tryptic soy agar and Sorbitol MacConkey agar or MacConkey agar plates (TSA, SMA, and MCA, Becton Dickinson Microbiology Systems).

Survival characteristics of E. coli O157:H7 in drinking water containing different amounts of rumen content and held at different temperatures: Tap water was mixed with a mixture of rumen content collected from three different beef cattle at a ratio of 100:1, 50:1, 25:1, 10:1, and 5:1 (ml:g), inoculated with a 5-strain mixture of ca. 10^6 *E. coli* O157:H7/ml (high inoculum) or 10^3 *E. coli* O157:H7 (low inoculum), and held at 8°C or 21°C. A 1-ml sample was obtained at appropriate sampling times and serially diluted, and then 0.1 ml from each dilution was plated onto Sorbitol MacConkey agar containing 50 µg nalidixic acid /ml (SMA-NA) and incubated at 37°C for 24 h. Colonies typical of *E. coli* O157:H7 (sorbitol-negative) were randomly picked for confirmation of *E. coli* by biochemical tests (API 20E miniaturized diagnostic test, bioMérieux Vitek, Hazelwood, MO.) and for confirmation of serogroup O157 by latex agglutination assay (Oxoid, Ogdensburg, N.Y.). When *E. coli* O157:H7 was not detected by direct plating, a selective enrichment (TSB with 50 µg nalidixic acid/ml) at 37ºC for 24 h was applied. Isolates of *E. coli* O157:H7 obtained at the end of some studies were analyzed by pulsed-field gel electrophoresis analysis according to the method we reported before to identify the dominant surviving stain (Brown, et al. 1997).

Chlorine and chlorine dioxide treatments: Standard chlorine solutions obtained from the HACH Company (Loveland, CO) were freshly diluted for each experiment in deionized water to the required concentration according to the method we reported before (Zhao, et al. 2001). The concentration of free chlorine in diluted chlorine solutions was determined with a Digital Titrator (HACH Co.). The *E. coli* O157:H7 suspension (1 ml) was added to 199 ml rumen-contaminated water containing different concentration of chlorine

solution (21°C) being stirred with a magnetic stir bar in a 500-ml Erlenmeyer flask. At predetermined sampling times; 1.0 ml of the treated bacterial suspension was removed and mixed with 9.0 ml of neutralizing buffer (Becton Dickinson Microbiology Systems,). Bacteria were serially (1:10) diluted in 0.1% peptone water and 0.1 ml of each dilution was surfaced-plated onto TSA-NA and SMA-NA in duplicate. The plates were held at 37°C for 24 h and presumptive *E. coli* O157:H7 colonies were counted and confirmed by the methods described above. Studies with chlorine dioxide were conducted according to similar procedures. All studies were done in duplicate.

Treatment with competitive inhibition bacteria: A mixture of five nalidixic-resistant strains of *E. coli* O157:H7 at 10^5 cfu/ml and a mixture of three strains of competitive inhibition bacteria (*E. coli* #271, #786 and #797, Zhao, et al. 1998) antagonistic to *E. coli* O157:H7 at 10^7 cfu/ml were inoculated into different flasks containing a mixture of water and rumen content at ratios of 100:1, 50:1, 25:1, 10:1, 5:1 and held at 21°C. A volume of 1 ml was removed daily or every other day and serially diluted in 0.1% peptone. A sample of 0.1 ml from each dilution was plated on the surface of SMA-NA plates in duplicate, and plates were incubated for 24 h at 37°C. Presumptive *E. coli* O157:H7 colonies were counted and confirmed by the methods described above. All studies were done in duplicate.

Ozone treatments: Ozone was produced by an laboratory scale ozone generator (Model H-50, Hess Machine International, Ephrata, PA) equipped with an oxygen concentrator (Model AS-12, AirSep, Buffalo, NY) and ozone concentrations (ppm) were measured by the indigo colorimeter method. Ozonated (22-24 ppm at 5°C) water was mixed with rumen content at ratios of 100:1, 50:1, 25:1, 10:1, and 5:1. MilliQ water (Milli-Q Synthesis A10, Millipore Corp. Brillerica, Mass) was used as the control. One ml of a mixture of 5 strains of *E. coli* O157:H7 (10^8 cfu) was mixed with 199 ml of the ozonated water with rumen content at 5°C and sampled from 0 to 20 min. At each sampling time, 1 ml was removed, and immediately mixed with 9 ml of neutralizing buffer and serially (1:10) diluted and a volume of 0.1 ml from each dilution tube was plated on SMA-NA and TSA-NA plates in duplicate. Plates were incubated at 37°C for 24 h. Presumptive *E. coli* O157:H7 colonies were counted and confirmed according to the method described above. All studies were done in duplicate.

Chemical treatments: Chemicals, including lactic acid (0.05-0.5%, Fisher Scientific, Fair Lawn, NJ), hydrogen peroxide (0.5%, Sigma Chemicals Inc. St. Louis, MO), sodium benzoate (0.1%, Fisher Scientific), acidic calcium sulfate (0.9-4.5%, Mionix Inc., Naperville, IL), octanoic acid (0.05-1.5%, Aldrich Chemicals Inc. Milwaukee, WI), butyric acid (0.5-4%, Aldrich Chemicals Inc), propionic acid (0.5-4%, Sigma Chemicals Inc.), caprylic acid (0.05%, Aldrich Chemicals, Inc.) and chlorine dioxide (10-1000 ppm, Aldrich Chemicals Inc.) were evaluated separately or as a combination. The chemicals were diluted to appropriate concentrations in MilliQ water (Milli-Q Synthesis A10, Millipore Corp.) initially tested with the pure cultures of *E. coli* O157:H7. The effective chemical or combination of different chemicals was further tested for their killing effect on *E. coli* O157:H7 in tap water containing rumen content at different ratios. The 5-strain mixture of *E. coli* O157:H7 (10^8 cfu/ml) was added, held at 21°C and sampled for

up to 120 min. Following chemical treatment, 1.0-ml was immediately diluted (1:1) in neutralizing buffer, serially diluted in neutralizing buffer, and plated onto SMA-NA, TSA-NA and TSA plates in duplicate. The plates were incubated at 37°C for 24 h for bacterial counts. When *E. coli* O157 was not detected by direct plating, a selective enrichment in TSB-NA was performed. Colonies growing on the surface of TSA-NA plates were counted as presumptive *E. coli* O157:H7. Presumptive colonies from the highest dilution were further confirmed as *E. coli* by biochemical testing (API 20E miniaturized diagnostic test, bioMérieux Vitek, Hazelwood, Mo) and O157 by a rapid agglutination assay (Oxoid, Ogdensburg, N.Y.). Combinations of chemicals effective in killing *E. coli* O157:H7 were further evaluated in water containing a mixture of feces collected from three beef cattle at a ratio of 20:1 (ml:g) according to the methods described above for treatment in water containing rumen content. All effective chemical combinations were further evaluated for their killing effects on *E. coli* O26:H11 and O111:NM using the same protocol described for studies on *E. coli* O157:H7. All studies were done in duplicate or triplicate and results are reported as averages.

Cattle selection and training: Twenty-one pregnant dairy heifers were selected to determine palatability of drinking water treated with different chemical combinations. Heifers generally exceeded 454 kg body weight. Prior to the study, heifers were trained to use electronic Calan doors which allow each animal access to a specific water treatment in an individual water trough. Access to all other water sources was restricted, and all water for heifers was provided through the Calan doors. Heifers were group fed in an area adjacent to the Calan doors. Heifers had access to individual free stalls and had access to an outside exercise paddock. Following adaptation to water consumption through the Calan doors, cows entered a three week experimental period.

Palatability assay of different chemical combinations: Cows were assigned to one of four groups. Three of the groups contained five cows each, the fourth group contained six cows. The groups were randomly assigned one of the four experimental treatments to which they were exposed for one week. Following the first experimental week all cows were given fresh water for a week to ensure that they were fully hydrated at the beginning of the next experimental week. This was necessary because water intake was negatively affected during the first experimental week. For the start of the second experimental week, groups were reassigned to treatments different from those during the first experimental week. Cattle were offered water ad libitum and daily consumption was determined for each heifer. Unconsumed water was weighed out each morning and troughs were emptied and fresh water and treatments added. Heifers were weighed at the end of the trial.

Data analysis: Data from cattle studies were analyzed using the General Linear Models procedure of SAS. Included in the statistical model were cow group, treatment and period. In addition, data collected during the control week between experimental weeks was used as a covariant to adjust for individual differences in intake while receiving the control treatment. Only data collected after cows had stabilized their intake following the first experimental week were used for covariant analysis. Analyses of total water intake

and water intake per metabolic body weight (MBW) were conducted. Paired T-tests were used for mean separation, and comparisons of means were only within significant F tests.

Results and Discussion

Survival of *E. coli* O157:H7 in water contaminated with rumen content at 8°C revealed that *E. coli* O157:H7 inoculated at 10^6 cfu/ml survived for 16, 6, 8, 3, and 5 weeks at tap water to rumen content ratios of 5:1, 10:1, 25:1, 50:1 and 100:1 (v/w), respectively.

At 21°C, results revealed that *E. coli* O157:H7 inoculated at 10^6 cfu/ml survived for 8, 15, 23, >56 and 24 weeks at water:rumen content ratios of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively. Survival of *E. coli* O157:H7 was considerably greater at 21°C than at 8°C. PFGE analysis of the isolates obtained at 56 weeks at 21°C revealed that strains E0122 (cattle isolate) and E0139 (deer isolate) were the dominant survivors. These results indicate that drinking water for cattle could maintain *E. coli* O157:H7 for long periods of time and thereby serve as an important vehicle of transmission on the farm. Hence, effective and practical interventions to eliminate/control *E. coli* O157:H7 in drinking water for cattle are needed.

Treatment of *E. coli* O157:H7 with competitive inhibition *E. coli* in water containing rumen content decreased *E. coli* O157:H7 by 0.2 to 0.7 $\log_{10}$ cfu/ml by day 16 at 21°C (Table 1), whereas *E. coli* O157:H7 increased by 0.6 to 1.0 $\log_{10}$ cfu/ml in the control (no competitive inhibition *E. coli*, Table 1). These results indicate that treatment of cattle drinking water with competitive inhibition *E. coli* controls growth of *E. coli* O157:H7 but has minimal effect on reducing *E. coli* O157:H7 populations. Hence, it is not an impactful approach for treating drinking water for cattle to control *E. coli* O157:H7.

Chlorine at 5 ppm in water immediately killed 10^6-10^7 *E. coli* O157:H7/ml to undetectable levels. However, the addition of rumen content to water at 100 parts water to 1 part rumen content or more (v/w) immediately neutralized the killing effect of free chlorine (Table 2).

Ozone at 22-24 ppm and 5°C in water with no rumen content effectively killed 10^6-10^7 *E. coli* O157:H7/ml (to undetectable level by direct plating method). However, adding rumen content to water at levels of 100 parts water to 1 part rumen content or more greatly decreased the antimicrobial activity of ozone (Table 3). Little to no *E. coli* O157:H7 inactivation occurred in 50 parts water to 1 part rumen content.

All chemicals, including lactic acid (0.05-0.5%), hydrogen peroxide (0.5%), sodium benzoate (0.1%), acidic calcium sulfate (0.9%), butyic acid (0.5-1.5%), propionic acid (0.5-4%), chlorine dioxide (10-100 ppm), and 0.05% caprylic acid, did not substantially reduce (<1.0 log/ml) *E. coli* O157:H7 within 20 min when tested individually in water containing rumen content (100:1) at 21°C. However, increasing the

concentration of butyric acid to ≥2% and caprylic acid to ≥0.1% resulted in substantial inactivation of *E. coli* O157:H7 within 20 minutes (data not shown). However, these higher concentrations of butyric acid and caprylic acid were offensive to smell.

A variety of combinations of chemicals at different concentrations were subsequently evaluated for *E. coli* O157:H7 inactivation of more than 5 log cfu/ml within 20 min in water containing large amounts of rumen content (10:1). Three combinations, including: (A) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid; (B) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate; and (C) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid at 21°C killed >5 log *E. coli* O157:H7/ml within 20 min in water containing rumen content at a ratio of 10:1 (v/w), (Table 4). A fourth chemical combination (D) containing 0.1% lactic acid, 0.9% acidic calcium sulfate and 100 ppm chlorine dioxide reduced *E. coli* O157:H7 populations by 2.6 log cfu/ml within 20 min and by 5.0 log within 120 min (Table 4).

These four chemical combinations were tested for their antimicrobial effect on *E. coli* O26:H11 and *E. coli* O111:NM in water containing large amounts of rumen content (10:1). Results revealed that three combinations (A, B and C) had the same antimicrobial activity (ca. 5 log reduction within 20 min at 21°C) on *E. coli* O26:H11 (Table 5) and *E. coli* O111:NM except for Treatment B which required 30 min for a 5 log cfu/ml reduction (Table 6). Combination D reduced *E. coli* 026:H11 and *E. coli* O111:NM populations within 20 min by 4.3 and 3.0 log cfu/ml, respectively (Tables 5 and 6).

Further evaluation of these four chemical combinations on their antimicrobial activity to *E. coli* O157:H7, O26:H11 and O111:NM was determined in water containing cattle feces at a ratio of 20:1 (v/w). Results revealed that combinations A, B and C killed (more than 5 log cfu/ml reduction) all three pathogens at 21°C within 30 min (Tables 4-6). Combination D reduced *E. coli* O157:H7, O26:H11 and O111:NM populations within 30 min by 3.5, 4.9, and 4.6 log cfu/ml, respectively (Tables 4-6).

Results of intake by dairy heifers of drinking water containing chemical combinations A or C are shown in Table 7. The average consumption amount of water (pH 7.3) for the control group was 30.3 L/d, for treatment group consuming 0.1% lactic acid and 0.9% acidic calcium sulfate (pH 2.1) was 18.6 L/d, for treatment group C consuming 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (pH 2.0) was 13.8 L, and for treatment group A 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (pH 2.0) was 15.1 L/d. Water treatments significantly depressed water intake. All water treatments were significantly different from the control, and there were no significant differences among water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow body weight (BW) was converted to MBW (metabolic body weight, $BW^{0.75}$), and intake of water per

MBW was calculated (Table 7). Treatment effects for water intake/MBW were similar to those for total water intake.

Conclusions

1. Depending on initial cell numbers present, *E. coli* O157:H7 can survive at room temperature (21°C) in water contaminated with rumen content for more than one year.

2. The killing effect of 5 ppm chlorine and 22-24 ppm ozone was neutralized by the addition of rumen content at 100 parts water to 1 part or more of rumen content.

3. Three chemical combinations, including: (A) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.05% caprylic acid; (B) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.1% sodium benzoate; and (C) 0.1% lactic acid, 0.9% acidic calcium sulfate and 0.5% butyric acid were highly effective at 21°C in reducing large cell numbers (10^5 cfu/ml) of *E. coli* O157:H7, O26:H11 and O111:NM to undetectable levels (by enrichment) within 30 minutes in water heavily contaminated with rumen content at a ratio of 10:1 (water:rumen content) or feces at a ratio of 20:1 (water:feces).

Table 1. *E. coli* O157:H7 counts in water with rumen content at 21°C treated with 3 strains of competitive inhibition *E. coli*

Water: rumen content ratio	*E. coli* O157:H7 (log_{10} cfu/ml) at day: 0	1	2	3	6	9	13	16
50:1 (control)	3.5	4.6	5.5	4.8	5.8	5.7	5.5	4.1
50:1 (treatment)	4.4	4.7	5.0	4.5	5.2	4.8	4.9	3.7
100:1 (control)	3.4	4.8	5.4	5.5	5.9	5.7	5.5	4.4
100:1 (treatment)	4.1	4.2	4.9	4.6	4.7	4.6	4.5	3.9

Table 2. *E. coli* O157:H7 counts in water with rumen content at 21°C treated with 5 ppm chlorine

Water: rumen content ratio	*E. coli* O157:H7 (log_{10} cfu/ml) at min: 0	1	5	10	20
Water (control)	<1.7	<1.7	<1.7	<1.7	<1.7
100:1	5.6	5.6	5.6	5.8	5.6
50:1	7.2	7.0	7.1	7.0	7.1
25:1	6.4	6.5	6.6	6.5	6.5
10:1	6.4	6.4	6.6	6.7	6.7

Table 3. *E. coli* O157:H7 counts in water with rumen content at 5°C treated with 22-24 ppm ozone

Water: rumen content ratio	*E. coli* O157:H7 (log_{10} cfu/ml) at min: 0	1	2	5	10	20
200:1	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7
100:1	5.2	5.4	5.5	5.2	4.5	4.8
50:1	6.2	6.2	6.4	6.4	6.5	6.3
20:1	5.6	5.2	5.1	5.1	5.4	5.2
Ozonated water only	<1.7	<1.7	<1.7	<1.7	<1.7	<1.7
Water only	5.7	5.4	5.4	5.3	5.6	5.7

Table 4: *E. coli* O157:H7 counts in water containing rumen content (10:1, v/w) or feces (20:1, v/w) treated with different chemical combinations at 21°C

Treatment	*E. coli* O157:H7 counts (log_{10} cfu/ml) at min							
	0	2	5	10	20	30	60	120
Rumen content contamination								
E. coli O157:H7 only (pH 8.2)	6.2	6.1	6.1	6.0	5.9	5.9	6.1	5.9
0.1% lactic acid+0.9% acidic calcium sulfate (pH 1.9)	5.7	5.3	4.4	3.9	2.8	2.5	2.2	1.5
0.5% butyric acid (pH 4.0)	5.9	5.9	5.9	5.8	5.7	5.8	5.6	5.7
0.05% caprylic acid (pH 7.8)	6.0	6.0	6.0	5.9	6.0	5.9	5.9	6.0
0.1% sodium benzoate (pH 8.2)	5.9	6.0	6.0	5.9	6.0	5.9	6.0	6.1
0.1% lactic acid+0.9% acidic calcium sulfate+0.5% butyric acid (pH 2.1)	5.8	4.2	+[a]	+	-[b]	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.1)	6.7	4.9	2.8	1.5	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.0)	5.2	-	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.1)	5.7	4.3	3.7	3.4	3.1	2.9	2.1	+
Fecal contamination								
E. coli O157:H7 only (pH 8.5)	6.1	6.1	6.1	6.0	6.1	6.1	6.1	6.2
0.1% lactic acid+0.9% acidic calcium sulfate (pH 2.2)	5.5	5.1	4.6	3.9	2.1	2.0	2.0	2.0
0.5% butyric acid (pH 4.5)	6.0	6.0	6.0	6.1	6.1	6.1	6.0	6.0
0.05% caprylic acid (pH 7.1)	6.0	5.6	4.3	2.3	2.0	2.0	2.0	1.7
0.1% sodium benzoate (pH 8.8)	5.7	5.6	5.4	5.6	5.5	5.4	5.5	5.6
0.1% lactic acid+0.9% acidic calcium sulfate+0.5% butyric acid (pH 2.3)	5.8	5.2	3.6	3.1	2.6	+	+	+
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.2)	5.7	4.0	2.0	1.7	+	+	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.2)	4.9	2.0	+	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.3)	5.5	3.0	2.7	2.5	2.5	2.0	1.7	1.7

[a]+, Positive by enrichment culture (<0.7 log_{10} cfu/ml)
[bc]–, Negative by enrichment culture

Table 5: *E coli* O26:H11 counts in water containing rumen content (10:1, v/w) or feces (20:1, v/w) treated with different chemical combinations at 21°C

Treatment	*E. coli* O26:H11 counts (log_{10} cfu/ml) at min:							
	0	2	5	10	20	30	60	120
Rumen content contamination								
E. coli O26:H11 only (pH 8.8)	5.5	5.6	5.5	5.4	5.4	5.5	5.5	5.5
0.1% lactic acid+0.9% acidic calcium sulfate (pH 2.2)	5.0	4.4	3.7	3.2	2.4	2.3	1.8	1.0
0.5% butyric acid (pH 4.4)	5.4	5.4	5.3	5.4	5.4	5.2	5.4	5.1
0.05% caprylic acid (pH 7.0)	5.3	5.4	5.2	5.4	5.3	5.4	5.4	5.4
0.1% sodium benzoate (pH 8.6)	5.6	5.5	5.4	5.5	5.5	5.5	5.4	5.5
0.1% lactic acid+0.9% acidic calcium sulfate+0.5% butyric acid (pH 2.3)	5.3	4.1	+[a]	+	-[b]	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.3)	5.2	5.1	3.6	1.7	+	+	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.3)	5.7	-	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.2)	5.5	5.1	2.6	1.6	1.2	1.2	+	+
Fecal contamination								
E. coli O26:H11 only (pH 7.4)	5.4	5.5	5.4	5.6	5.5	5.4	5.4	5.5
0.1% lactic acid+0.9% acidic calcium sulfate (pH 2.1)	5.4	4.7	4.7	4.5	4.0	2.0	1.9	1.5
0.5% butyric acid (pH 4.1)	5.5	5.4	5.4	5.4	5.5	5.2	5.2	5.0
0.05% caprylic acid (pH 5.6)	5.5	5.4	5.5	5.5	5.3	5.2	5.2	5.0
0.1% sodium benzoate (pH 7.8)	5.5	5.4	5.5	5.4	5.5	5.4	5.5	5.4
0.1% lactic acid+0.9% acidic calcium sulfate+0.5% butyric acid (pH 2.1)	5.4	3.3	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.1)	5.3	+	+	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.0)	3.9	1.7	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.1)	4.9	1.7	+	+	+	-	-	-

[a]+, Positive by enrichment culture (<0.7 log_{10} cfu/ml)
[b]–, Negative by enrichment culture

Table 6: *E. coli* O111:NM counts in water containing rumen content (10:1, v/w) or feces (20:1, v/w) treated with different chemical combinations at 21°C

Treatment	*E. coli* O111:NM counts (log_{10} cfu/ml) at min							
	0	2	5	10	20	30	60	120
Rumen content contamination								
E. coli O111:NM only (pH 8.7)	5.8	5.9	5.7	5.7	5.8	5.8	5.9	5.8
0.1% lactic acid+0.9% acidic calcium sulfate (pH 2.2)	5.5	5.4	4.2	2.3	2.1	2.0	1.6	1.0
0.5% butyric acid (pH 3.8)	5.5	5.5	5.5	5.6	5.6	5.4	5.5	5.6
0.05% caprylic acid (pH 5.7)	5.6	5.5	5.7	5.5	5.8	5.6	5.4	5.5
0.1% sodium benzoate (pH 8.6)	5.6	5.6	5.5	5.6	5.6	5.5	5.4	5.5
0.1% lactic acid+0.9% acidic calciumsulfate+0.5% butyric acid (pH 2.3)	5.7	5.0	2.8	+[a]	+	-[b]	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.3)	5.7	5.3	4.4	3.6	2.1	+	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.2)	4.4	+	+	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.3)	5.8	5.1	3.3	3.0	2.8	2.6	2.5	2.0
Fecal contamination								
E. coli O111:NM only (pH 7.7)	5.6	5.7	5.7	5.6	5.6	5.6	5.7	5.7
0.1% lactic acid+0.9% acidic calcium sulfate (pH 2.2)	4.5	3.1	1.9	1.7	1.7	1.7	1.7	1.4
0.5% butyric acid (pH 4.1)	5.6	5.6	5.5	5.6	5.6	5.6	5.6	5.6
0.05% caprylic acid (pH 5.5)	5.7	5.5	5.7	5.5	5.6	5.7	5.5	5.4
0.1% sodium benzoate (pH 7.6)	5.6	5.6	5.6	5.7	5.6	5.5	5.5	5.6
0.1% lactic acid+0.9% acidic calcium sulfate+0.5% butyric acid (pH 2.0)	5.4	3.3	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+0.1% sodium benzoate (pH 2.1)	5.7	4.5	1.5	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate +0.05% caprylic acid (pH 2.0)	5.5	2.3	-	-	-	-	-	-
0.1% lactic acid+0.9% acidic calcium sulfate+100 ppm chlorine dioxide (pH 2.0)	5.3	2.7	2.6	1.9	+	+	+	-

[a]+, Positive by enrichment culture (<0.7 log_{10} cfu/ml)
[b]–, Negative by enrichment culture

Table 7. Effect of drinking water treatments on water intake by dairy heifers.

Treatment	Water intake, L/d	Intake/MBW[1], kg/kg
Control	30.3 [a]	26.3 [a]
0.1% lactic acid + 0.9% acidic calcium sulfate	18.6 [b]	16.3 [b]
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (Treatment C)	13.8 [b]	11.9 [b]
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (Treatment C)	15.1 [b]	12.6 [b]

[1] Metabolic body weight
[a,b] Means within columns with different superscripts are different ($P < 0.01$)

References

Al-Saigh, H., C. Zweifel, J. Blanco, J. E. Blanco, M. Blanco, M. A. Usera, and R. Stephan. 2004. Fecal shedding of *Escherichia coli* O157:H7, *Salmonella*, and C*ampylobacter* in Swiss cattle at slaughter. J. Food Prot. 67:679-684.

Bach, S. J., T. A. McAllister, G. J. Mears, and K. S. Schwartzkopf-Genswein. 2004. Long-haul transport and lack of preconditioning increases fecal shedding of *Escherichia coli* and *Escherichia coli* O157:H7 by calves. J. Food Prot. 67:672-678.

Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and enterobacteriaceae are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. J. Food Prot. 67:646-650.

Castillo, A., K. S. McKenzie, L. M. Lucia, and G. R. Acuff. 2003. Ozone treatment for reduction of *Escherichia coli* O157:H7 and *Salmonella* serotype *typhimurium* on beef carcass surfaces. J. Food Prot. 66:775-779.

Callaway, T. R., R. C. Anderson, T. S. Edrington, R. O. elder, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2003. Preslaughter intervention strategies to reduce food-borne pathogen in food animals. J. Anim. Sci. 81:17-23.

Dev, V. J., M. Main, and I. Gould. 1991. Waterborne outbreak of *Escherichia coli* O157. Lancet 337:1412.

Faith, N. G., J. A. Shere, R. Brosch, K. W. Arnold, S. E. Ansay, M. S. Lee, J. B. Luchansky, and C. W. Kaspar. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl. Environ. Microbiol. 62:1519-1525.

Finch, G. R., D. W. Smith, and M. F. Stiles. 1988. Dose response of *Escherichia coli* in ozone demand-free phosphate buffer. Water Res. 22:1563-1570.

Friedman, M. S., T. Roels, J. E. Koehler, L. Feldman, W. F. Bibb, P. A. Blake. 1999. *E. coli* O157:H7 outbreak associated with an improperly-chlorinated swimming pool. Clin. Infect. Dis. 29:298-303.

Harmon, B. G., C. A. Brown, S. Tkalcic, P. O. E. Mueller, A. Parks, A. V. Jain, T. Zhao, and M. P. Doyle. 1999. Fecal shedding and rumen growth of *Escherichia coli* O157:H7 in fasted calves. J. Food Prot. 62:574-579.

LeJeune, J. T., T. E. Besser, and D. D. Hancock. 2001. Cattle water troughs as reservoirs of Escherichia coli O157. Appl. Environ. Microbiol. 67:3053-3057

Paunio, M., R. Pebody, M. Keskim·ki, M. Kokki, P. Ruutu, S. Oinonen, V. Vuotari, A. Siitonen, E. Lahti, and P. Leinikki. 1999. Swimming-associated outbreak of *Escherichia coli* O157:H7. Epidemiol. Infect. 122:1-5.

Restaino, L., E. W. Frampton, J. B. Hemphill, and P. Palnikar. 1995. Efficacy of ozonated water against various food-related microorganisms. 61:3471-3475.

Rice, E. W., R. M. Clark, and C. H. Johnson. 1999. Chlorine inactivation of *Escherichia coli* O157:H7. Emerg. Infect. Dis. 5: 461-463.

Swerdlow, D. L., B. A. Woodruff, and R. C. Brady. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. 117:812-819.

Tuttle, J., T. Gomez, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin. 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol. Infect. 122:185-192.

Venkitanarayanan, K., T. Zhao and M. P. Doyle. 1999. Inactivation of *Escherichia coli* O157:H7 by combinations of GRAS chemicals and temperature. Food Microbiol 16:75-82.
Venkitanarayanan, K., G. O. Ezeike, Y. C. Hung, and M. P. Doyle. 1999. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. 65:4276-4279.

Venkitanarayanan, K., C. M. Lin, H. Bailey, and M. P. Doyle. 2002. Inactivation of *Escherichia coli* O157:H7, *salmonella enteritidis*, and *listeria monocytogenes* on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide. J. Food Prot. 65:100-105.

Wade, W. N., A. J. Scouten, K. H. McWatters, R. L. Wick, A. Demirci, W. F. Fett, and L. R. Beuchat. 2003. Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. J. Food Prot. 66:44-51.

Wang, G. and M. P. Doyle. 1998. Survival of enterohemorrhagic Escherichia coli O157:H7 in water. J. Food Prot. 61:662-667.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61:1290-1293.

Zhao, T., M. P. Doyle, P. Zhao, P. Blake, and F. M. Wu. 2001. Evaluation of the efficacy of chlorine on the inactivation of *Escherichia coli* O157:H7 in water. J. Food Protect. 64:1607-1609.

Zhao, T., M. P. Doyle, B. G. Harmon, C. Brown, P O Eric Mueller, A. H. Parks. 1998. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. J. Clin. Microbiol. 36:641-647.