Center for Food Safety, University of Georgia, Griffin, GA (http://www.griffin.uga.edu/cfs)
2003 Highlights

WATER

SURVIVAL AND CONTROL OF *E. COLI* O157:H7 IN DRINKING WATER FOR CATTLE

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A study published in 1999 by the Centers for Disease Control and Prevention estimated that *E. coli* O157:H7 accounts for more than 73,000 cases of foodborne illness each year in the United States. Although a variety of different foods have been implicated as vehicles of infection, most *E. coli* O157:H7 foodborne outbreaks are associated with the consumption of undercooked ground beef. In 2002 alone, approximately 20 million pounds of ground beef contaminated with *E. coli* O157:H7 were recalled by the United States Department of Agriculture. Researchers have determined previously that *E. coli* O157:H7 can survive in bovine feces and water contaminated with cattle feces for long periods of time and retain their ability to produce verotoxins. Cattle are a principal reservoir of *E. coli* O157:H7 and their drinking water at the farm is an important source for their transmission. The objectives of this project were to evaluate the survival characteristics of *E. coli* O157:H7 in water after it is contaminated by rumen content, a site in cattle where *E. coli* O157:H7 resides, and to develop methods to control *E. coli* O157:H7 in contaminated water containing rumen fluid.

Survival of *E. coli* O157:H7 (a 5-strain mixture, including E0018, E009, 932, E0122, and E0139) in water contaminated with rumen content at different ratios, cell numbers and temperatures were determined. At 8ºC, results revealed that *E. coli* O157:H7 at a high level of inoculum (10^5 cfu/ml) survived for 16, 6, 8, 3, and 5 weeks at ratios (tap water to rumen content) of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively, and at low level of inoculum (10^2 cfu/ml) for 2, 3, 1, 1, and 1 weeks at ratios of 5:1, 10;1, 25:1, 50:1 and 100:1, respectively. However, at 21ºC, results revealed that *E. coli* O157:H7 at a high level of inoculum survived 8, 15, 23, >56 and 24 weeks at contamination ratios of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively, and at a low level of inoculum survived 8, 11, 10, 11, and 10 weeks at ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively. Results of studies at 8ºC indicate the more concentrated the rumen content, the greater the survival of *E. coli* O157:H7. On the contrary, survival trends at 21ºC were greater when the rumen content was more dilute. With equivalent cell numbers of *E. coli* O157:H7 inoculated, the survival of the pathogen was much greater at 21ºC than at 8ºC. DNA profile analysis of the isolates obtained at 56 weeks when held at 21ºC revealed that E0122 (cattle isolate), 932 (meat isolate) and E0139 (deer isolate) were the dominant surviving strains.

Treatment of water with chlorine revealed that free chlorine at 1 ppm (the concentration present in tap water) is sufficient to kill up to 10^7 cfu *E. coli* O157:H7/ml within minutes. However, results from studies with tap water contaminated with different levels of rumen content, which included 10:1, 25:1, 50:1 and 100:1, revealed that the concentration of free chlorine was eliminated within minutes. Free chlorine (5 ppm) was added to tap water contaminated with rumen content at a ratio of 10:1, 25:1, 50:1 and 100:1. *E. coli* O157:H7 cell numbers ranging from 10^5 to 10^7 cfu/ml were added at 21ºC and sampled at 0, 1, 2, 5, 10 and 20 minutes for enumeration of *E. coli* O157:H7. Results revealed there was no change in *E. coli* O157:H7 counts within 2 min for any of the contamination ratios evaluated. For samples with a contamination ratio of 100:1, *E. coli* O157:H7 counts at 5, 10 and 20 min were reduced by 0.3, 1.1 and 3.8 $\log_{10}$ cfu/ml, respectively; at 25:1, *E. coli* O157:H7 counts were reduced by 0, 0.2 and 1.1 cfu/ml, respectively; and at 10:1, *E. coli* O157:H7 counts were not reduced within 20 min.

The effect of ozonated water on *E. coli* O157:H7 was also evaluated. The concentration of ozone in water for all determinations ranged from 22-24 ppm, and the temperature was 5°C. The *E. coli* O157:H7 inoculum ranged from $10^{5.2}$ to $10^{6.7}$ cfu/ml. Four to 6 trials have been completed for studies with water to rumen content at ratios of 50:1 and 100:1. Results revealed that 22-24 ppm of ozone in water with no rumen content killed up to 10^5 *E. coli* O157:H7/ml within 1 min. However, the influence of ozone in killing *E. coli* O157:H7 was related to the amount of rumen content present in water. At a water to rumen content ratio of 20:1 and 50:1, a contact time of up to 20 min reduced *E. coli* O157:H7 by only 0.4 $\log_{10}$ cfu/ml. Inconsistent results were obtained for studies done with ozone in water containing rumen content at a ratio of 100:1. For three of the 6 trials, *E. coli* O157:H7 populations were reduced by greater than 4 $\log_{10}$ cfu/ml within 1 min of exposure to ozone. For one trial, a greater than 4 $\log_{10}$ cfu/ml reduction occurred after 5 min or more exposure time. Interestingly, there was no substantial reduction of *E. coli* O157:H7 for two trials, i.e., ca. 0.7 $\log_{10}$ cfu/ml after 10 min or exposure for one trial and no inactivation of *E. coli* O157:H7 after 20 min of exposure for the other trial. These results indicate that the effect of ozone on *E. coli*

O157:H7 is related to the amount of rumen content in water. Contamination of water with rumen content at a ratio more concentrated than 50:1 neutralizes the killing effect of 22-24 ppm ozone.

Treatment of water with probiotic *E. coli* (a mixture of 3 strains, including #271, #786 and #797, all of which produce metabolites antagonistic to *E. coli* O157:H7) to inactivate *E. coli* O157:H7 was studied at 21°C for a period of 15 days. Experimental conditions included water to rumen content ratios of 50:1 and 100:1, a 5-strain mixture of *E. coli* O157:H7 at 10^3 cfu/ml and probiotic *E. coli* at 10^6 cfu/ml. The studies were repeated five times. Within 15 days, the population of *E. coli* O157:H7 in rumen content-contaminated water increased (up to 2.5 $\log_{10}$ cfu/ml), whereas the population of *E. coli* O157:H7 treated with probiotic *E. coli* decreased from 0.2 to 2.5 $\log_{10}$ cfu/ml). Greater *E. coli* O157:H7 inactivation occurred in water to rumen content at a ratio of 100:1 than at 50:1.
A group of chemicals, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, sodium hydroxide, caprylic acid, propionic acid, and butyric acid were tested for their effect on the killing of *E. coli* O157:H7 individually or as a combination. Of these, a combination of lactic acid (0.1-0.5%), acidic calcium sulfate (0.5.0.9%) and chlorine dioxide (50-100 ppm) or lactic acid (0.1-0.5%), acidic calcium sulfate (0.9%) and caprylic acid (0.1-0.5%) at 21ºC effectively killed > 5.0 $\log_{10}$ *E. coli* O157:H7 within 2 min in water heavily contaminated with rumen content at a ratio of 10:1. Additional experiments are underway to identify the most cost effective combinations for on-farm use.

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