

BEEF OR PORK

SURVIVAL AND CONTROL OF *E. COLI* O157:H7 IN DRINKING WATER FOR CATTLE (P. Zhao, M. P. Doyle, and T. Zhao)

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 8°C, 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 21°C, 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 8°C indicate the more concentrated the rumen content, the greater the survival of *E. coli* O157:H7. On the contrary, survival trends at 21°C 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 21°C than at 8°C. DNA profile analysis of the isolates obtained at 56 weeks when held at 21°C 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 21°C 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 10³ cfu/ml and probiotic *E. coli* at 10⁶ 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 log₁₀ cfu/ml), whereas the population of *E. coli* O157:H7 treated with probiotic *E. coli* decreased from 0.2 to 2.5 log₁₀ 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 21°C effectively killed > 5.0 log₁₀ *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.

VIABILITY OF ACID-ADAPTED *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF TREATED WITH ACIDIC CALCIUM SULFATE (L. R. Beuchat and A. J. Scouten)

Exposure of microorganisms to sublethal stress can result in greater resistance of cells upon subsequent exposure to the same or unrelated stress. Acid adapted or acid shocked *Escherichia coli* O157:H7 has been shown to have increased resistance to heat, salt, and acidic pH. The type of acidulant used to achieve a given pH can influence the rate of inactivation of *E. coli* O157:H7 as well as its sensitivity upon subsequent exposure to acid or other stress environments. The effectiveness of treatment of beef carcasses with organic acids for the purpose of reducing or eliminating surface microflora, including *E. coli* O157:H7 and other pathogens, varies with type, concentration and temperature of acid, the presence of surfactants, contact time, application pressure, tissue type, and sensitivity of the microorganism. The potential for *E. coli* O157:H7 surviving acid rinse treatment of beef carcasses to exhibit increased tolerance to acidic environments or refrigeration temperatures to which processed beef may be exposed raises a concern about the impact of organic acid sanitizers on safety risks. Supplementation of ground beef with acidic calcium sulfate (ACS), the basis for a commercial food additive called Safe₂O™, which contains calcium hydroxide, sulfuric acid, and calcium sulfate, has been suggested as an intervention to control the growth of spoilage and pathogenic bacteria. Claims are that ACS kills bacteria at levels nearly equal to irradiation and continues to inhibit microbial growth and recontamination long after treatment. Bacterial kill in excess of 5 log₁₀ is claimed. The effects of ACS on survival of acid-adapted *E. coli* O157:H7 in refrigerated ground beef have not been reported.

The objectives of this study were to determine if treatment of three strains of *E. coli* O157:H7 in broth acidified with lactic acid, acetic acid, or ACS results in a change in tolerance of cells upon exposure to a second acidic environment and to determine if *E. coli* O157:H7 grown on an agar medium at pH 4.5 achieved using ACS as an acidulant changes in its ability to survive in ACS-treated ground beef. Differences in tolerance to acidic environments were observed among strains but the level of tolerance was not affected by the acidulant to which cells had been exposed. Cells of *E. coli* O157:H7 adapted to grow on tryptic soy agar acidified to pH 4.5 with ACS were compared to cells grown at pH 7.2 in the absence of ACS for their ability to survive after inoculation into ground beef treated with ACS, as well as untreated beef. The number of ACS-adapted cells recovered from ACS-treated beef was significantly ($\alpha = 0.05$) higher than the number of control cells recovered from ACS-treated beef during the first 3 days of a 10-day storage period at 4°C, suggesting that ACS-adapted cells are initially more tolerant than unadapted cells to reduced pH in ACS-treated beef. Regardless of treatment of ground beef with ACS or adaptation of *E. coli* O157:H7 to ACS before inoculating ground beef, the pathogen survived in high numbers.

SEROPREVALENCE OF *TOXOPLASMA* AND *NEOSPORA* IN CATTLE AND SWINE (Y. R. Ortega, G. Saavedra, and P. Torres)

Toxoplasma and *Neospora* are apicomplexans that can infect a variety of animals, some of which are used for human consumption. *Toxoplasma* also infects humans and can cause encephalitis. In pregnant women, fetal toxoplasmosis can result in abortions or produce birth defects, blindness, encephalitis, and chorioretinitis. Infection can be acquired by ingestion of *Toxoplasma* oocysts which are excreted in the feces of infected felines, ingestion of raw meats containing viable tissue cysts, or by organ transfusions. *Neospora* causes abortion in cattle.

Sera from 290 pigs and 329 cattle from U.S. and Peru were collected from slaughterhouses. Parasite-specific antibody responses to *Neospora caninum* and *Toxoplasma gondii* antigens were detected using the immunoblot assay.

Of the serum samples from Peru, 38/137 (28%) of the porcine and 127/253 (50%) of the bovine sera were positive for *T. gondii*, and 3 (2%) of pigs and 20 (8%) of cows were positive for *N. caninum*. In the U.S., 33/76 (43%) of cows and 23/153 (15%) of pigs were positive for *T. gondii* and 10 (13%) of cows and 1 (0.6%) of pigs were positive for *N. caninum*. Antibodies to both *N. caninum* and *T. gondii* were found in 12 (5%) of 253 cows and 3 (2%) of 137 pigs from Peru. In the US, 5/76 (7%) of cows and 0/153 (0%) in swine sera were positive for both parasites.

**FATE OF *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES*,
AND *SALMONELLA* SPP. IN REDUCED SODIUM HOME-STYLE BEEF JERKY
(M. A. Harrison)**

Interest in low-sodium food products necessitates re-examination of home preservation processes relying in part on salt for antimicrobial effects. The fates of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. in reduced sodium home-style beef jerky was determined using different salt levels in ground or whole beef strips. Samples were either dried in a 60°C dehydrator or heated to an internal temperature of 71.1°C prior to drying in a 60°C dehydrator. Populations were determined at time 0 and 2 h intervals until dry. Population reductions were greater in ground beef with non-reduced salt levels compared to that with reduced salt levels, and in most cases, greater reduction (1.0 – 1.5 log₁₀) was observed for ground beef strips heated prior to drying. For dried whole jerky strips, there generally were no significant differences (p > 0.05) in pathogen populations between the non-reduced and reduced salt marinade in the end product. The results from this study support the importance of the antimicrobial effect of sodium chloride in particular products on the pathogens used in this experiment.

**COMPARISON OF USDA CULTURE PROCEDURE AND POLYMERASE CHAIN REACTION (PCR)
DETECTION OF *LISTERIA MONOCYTOGENES* ON DELI MEATS
(C.-M. Lin, L. Zhang, and M. P. Doyle)**

A polymerase chain reaction (PCR) assay (BAX[®], DuPont, Wilmington, DE) for detecting *Listeria monocytogenes* in meat products has been adopted by the US Department of Agriculture (USDA). In addition, USDA-FSIS has promoted using a large sampling size such as an entire package for detecting *L. monocytogenes* in meat products. This study was conducted to compare the BAX-PCR assay and the USDA culture enrichment method to detect *L. monocytogenes* on deli meats contaminated during slicing by a slicer blade inoculated with listeriae. Two types of deli meat, salami and bologna, were tested. The salami had a low pH and moisture content and the bologna contained antimicrobials to *L. monocytogenes*, hence neither meat would support the growth of listeriae. A five-strain (Bil Mar, Coleslaw, Plantation, Jalisco, OM) mixture of *L. monocytogenes* at 10² cfu was inoculated onto the surface of the blade of a commercial slicer. A commercial log of meat product was sliced in its entirety and five slices per package were packed, vacuum-sealed, stored at 4°C, then sampled at 1, 30, 60, and 90 days post-slicing. Meat sliced before the blade was inoculated with *L. monocytogenes* was used as the negative control. Meat from eight negative-control packages was inoculated with 10¹ or 10² cfu of *L. monocytogenes* to serve as positive controls. Studies with each type of meat were done in duplicate. A total of 132 samples were analyzed at each sampling date. The entire package of deli meat was stomached with UVM broth and incubated at 30°C for 24 h. For the USDA culture enrichment procedure, 0.1 ml of UVM enrichment culture was transferred into Fraser broth then incubated and streaked onto modified Oxford agar (MOX) plates, and a loopful of the enrichment culture was streaked directly onto MOX plates. Presumptive isolates of *L. monocytogenes* obtained from either culturing in Fraser broth or by direct plating onto MOX were confirmed by hemolysis on horse blood agar plates, then biochemical assay with API strips (bioMérieux Inc., Hazelwood, MO) and BAX-PCR. For the BAX-PCR assay, 0.1 ml of the UVM-enrichment culture was transferred into BAX-*Listeria* enrichment broth.

PCR-positive samples were further assayed by streaking the BAX-*Listeria* broth onto MOX and horse blood agar plates, then isolates were confirmed with API strips. A three-tube most probable number (MPN) method was used to enumerate *L. monocytogenes* populations in positive samples. There were very few *Listeria*-positive samples. For the two trials for each type of meat, at most two meat packages were confirmed *L. monocytogenes*-positive on the first sampling day. The number of *Listeria*-positive samples declined during storage. No sample was confirmed *Listeria*-positive at 30 and 60 days for either salami or bologna; however, one 90-day sample of salami in the second trial was confirmed *L. monocytogenes*-positive. None of the negative controls was positive and *L. monocytogenes* in the positive controls inoculated at the 10^1 cfu level could not be recovered at 30 and 60 days on either salami or bologna. Based on MPN, *L. monocytogenes* populations were very low (from < 0.06 to 0.75 MPN/g) and decreased gradually during storage, even in positive control samples. The number of *L. monocytogenes*-positive samples determined by PCR or culture enrichment was similar. Most of the *Listeria*-positive samples were detected by both PCR and culture enrichment; however, some samples were determined *Listeria*-positive by PCR but not by culture enrichment and vice versa. Our results indicate that the ability of the BAX-PCR assay and the USDA culture enrichment to detect small cell numbers of *L. monocytogenes* on salami and bologna was similar, and that neither bologna with antimicrobial agents nor salami supported the growth of *L. monocytogenes* throughout 90 days of storage at 4°C.

