

E. Coli

Fates of Acid-Resistant and Non-Acid-Resistant Shiga Toxin-Producing *Escherichia coli* Strains in Ruminant Digestive Contents in the Absence and Presence of Probiotics

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Significance: The rumen compartment could be a target for intervention strategies that could limit Shiga toxin-producing *Escherichia coli* (STEC) survival and eliminate induction of acid resistance mechanisms in order to decrease the number of STEC cells reaching the hindgut and thus food contamination.

This study investigated whether acid resistance confers an ecological advantage to Shiga toxin-producing *Escherichia coli* (STEC) strains in ruminant digestive contents and whether acid resistance mechanisms are induced in the rumen compartment. Acid-resistant STEC survived at higher rates during prolonged incubation in rumen fluid than acid-sensitive STEC and they resisted the highly acidic conditions of the abomasum fluid, whereas acid-sensitive strains were killed. Transit through the rumen contents allowed acid-sensitive strains to survive in the abomasum fluid at levels similar to those of acid-resistant STEC. Supplementation with the probiotic *Saccharomyces cerevisiae* CNCM I-1077 or *Lactobacillus acidophilus* BT-1386 led to killing of all of the strains tested during prolonged incubation in the rumen contents. *S. cerevisiae* did not limit the induction of acid resistance in the rumen fluid.

Behavior of *Escherichia coli* O157:H7 on Damaged Leaves of Spinach, Lettuce, Cilantro, and Parsley Stored at Abusive Temperatures

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Significance: The damaged portion of a leafy green is a distinct growth niche that elicits different microbial responses in the various types of leafy greens.

The behavior of *Escherichia coli* O157:H7 on damaged leaves of baby Romaine lettuce, spinach, cilantro, and parsley stored at three abusive temperatures (8, 12, and 15°C) were compared. The damaged portions of leaves were inoculated with approximately 10⁵ CFU *E. coli*/leaf. The pathogen grew on damaged spinach leaves held for 3 days at 8 and 12°C (P<0.05), with the population increasing by 1.18 and 2.08 log CFU/leaf, respectively. *E. coli* did not grow on damaged Romaine leaves at 8 or 12°C, but growth was observed after 8 h of storage at 15°C, with an increase of <1.0 log. Growth of *E. coli* on Romaine lettuce held at 8 or 12°C was enhanced when inocula were

suspended in 0.05% ascorbic acid. Damaged cilantro and Italian parsley leaves held at 8°C for 4 days did not support the growth of *E. coli*. Behavior of the pathogen in leaf extracts differed from behavior on the damaged tissue.

Reduction of *Escherichia coli* O157:H7 in Fresh Spinach, Using Lactic Acid Bacteria and Chlorine as a Multihurdle Intervention

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Significance: The industry standard chlorine wash may be more effective when applied in combination with lactic acid bacteria.

A 12-day shelf life study was conducted at 7°C to determine whether *Escherichia coli* O157:H7 on spinach can be controlled effectively by selected strains of lactic acid bacteria (LAB) alone or in combination with chlorine as a multihurdle intervention, a combination of LAB and chlorine which was applied to spinach as a rinse and evaluated in comparison to LAB alone and chlorine and water rinses. All spinach samples were packaged, held in a display case, and tested for *E. coli* on days 0, 1, 3, 6, 9, and 12. Survival of LAB throughout the shelf life also was determined. Significant reductions in pathogen populations were achieved by water ($P=0.0008$), LAB ($P<0.0001$), chlorine ($P<0.0001$), and multihurdle ($P<0.0001$) treatments when compared with controls. The multihurdle treatment produced the greatest reduction from control populations (1.91 log CFU/ml). This reduction was significantly greater than that achieved with water ($P<0.0001$), LAB ($P=0.0025$), and chlorine ($P<0.0001$).

Evaluation of a Direct-Fed Microbial Product Effect on the Prevalence and Load of *Escherichia coli* O157:H7 in Feedlot Cattle

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Significance: The direct-fed microbial tested would not be an effective preharvest intervention against *E. coli* O157:H7.

The efficacy of a direct-fed microbial (DFM) consisting of *Bacillus subtilis* strain 166 as an antimicrobial intervention strategy for the reduction of prevalence and load of *E. coli* O157:H7 in feces and on hides of feedlot cattle was evaluated in 256 cattle ($n=526$) divided among 16 feedlot pens. Half of the pens received the DFM, and the other half did not. Hide and fecal samples were collected from each animal on days 28, 63, and 84 of the feeding trial. Over the course of the 84-day feeding period, there were no significant differences observed between treatments for either hide or fecal prevalence of *E. coli*, or for the percentage of animals that were shedding *E. coli* at high levels (≥ 200 CFU/g) in their feces or harboring *E. coli* at high levels (≥ 40 CFU/cm²) on their hides.

Listeria

Antilisterial Effects of Gravinol-S Grape Seed Extract at Low Levels in Aqueous Media and Its Potential Application as a Produce Wash

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Significance: Grape seed extract has the potential as a natural control of *Listeria* spp. on low-complexity foods such as tomatoes.

This study sought to characterize the antilisterial effects of a commercial grape seed extract (GSE) preparation (Gravinol-S) alone at much lower concentrations (0.00015 to 0.125%) in aqueous solution and to test its possible use as an antimicrobial wash for fresh produce surfaces. The MICs of GSE against *Listeria monocytogenes* Scott A and *Listeria innocua* ATCC 33090 were as low as 50 and 78 $\mu\text{g ml}^{-1}$, respectively. At sub-MICs and after only 2 min of exposure, treatment with GSE caused rapid permeabilization and clumping of *L. innocua*, results that we confirmed for *L. monocytogenes* using fluorescence microscopy and Live/Dead staining. At higher concentrations (0.125%), GSE reduced viable cell counts for *L. monocytogenes* by approximately 2 log units within 2 min on tomato surfaces.

Salmonella

Thermal Inactivation and Postthermal Treatment Growth during Storage of Multiple Salmonella Serotypes in Ground Beef as Affected by Sodium Lactate and Oregano Oil

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Significance: Lactate and oregano oil may be used to render *Salmonella* spp. more susceptible to the lethal effect of heat and to inhibit growth of *Salmonella* spp. that survives heat treatments.

This study investigated the heat resistance of *Salmonella* in raw ground beef in both the absence and presence of sodium lactate (NaL), oregano oil, and in combinations, and determined their bactericidal or bacteriostatic activities during postthermal treatment storage at 15°C. A cocktail of 8 serotypes of *Salmonella* spp. was inoculated into ground beef supplemented with NaL (1.5% and 3%) and/or oregano oil (0.5% and 1%) to obtain approximately 8 log CFU/g. The ground beef samples (3g) were vacuum-packed and heated at 60, 65, or 71°C in a circulating water bath to inactivate approximately 5-6 log CFU/g of the pathogen, and then stored at 15°C for 15 and 30 d. Results showed that addition of oregano oil increased the inactivation rate of *Salmonella* spp. at lower temperatures, whereas addition of NaL alone exhibited a protective effect against lethality and decreased the rate. Addition of combinations of oregano oil and NaL overcame this protective effect. During subsequent posttreatment storage for 15 d, *Salmonella* populations in the controls and in samples containing 0.5% oregano (60 and 65°C) or 1% oregano oil (60°C) increased to 4.5-6 log CFU/g.

Foodborne Pathogens

Quantitative Microbial Risk Assessment for Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in Leafy Green Vegetables Consumed at Salad Bars

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Significance: An integration of modeling pathogen growth in the supply chain of fresh leafy vegetables destined for restaurant salad bars was included in this study.

A quantitative microbial risk assessment for Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes infection from consumption of leafy green vegetables in salad from salad bars using time-temperature profiles in the chilled supply chain and one particular restaurant with a salad bar was conducted. A second-order Monte Carlo risk assessment model was constructed (using @Risk) to estimate the public health effects. The temperature in the studied cold chain was well controlled below 5°C. Growth of E. coli and Salmonella was minimal. Growth of L. monocytogenes was considerably greater (194%). Based on first-order Monte Carlo simulations, the average number of cases/year in The Netherlands associated the consumption of leafy greens in salads from salad bars was 166, 187, and 0.3 for E. coli, Salmonella, and L. monocytogenes, respectively. The ranges of the average number of annual cases as estimated by second-order Monte Carlo simulation were 42-551 for E. coli, 81-281 for Salmonella, and 0.1-0.9 for L. monocytogenes.

Norovirus

Comparison between Quantitative Real-Time Reverse Transcription PCR Results for Norovirus in Oysters and Self-Reported Gastroenteric Illness in Restaurant Customers

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Significance: Detection of high levels of norovirus RNA in oysters is indicative of a significantly elevated health risk.

This study compares norovirus RNA detection in Pacific oysters (*Crassostrea gigas*) by quantitative real-time reverse transcription PCR (RT-PCR) and human health risk. Self-reported customer complaints of illness in a restaurant setting were compared with presence and levels of norovirus as determined by real-time RT-PCR for the batch of oysters consumed. No illness was reported for batches consistently negative for norovirus by real-time RT-PCR. Norovirus was detected in some batches for which no illness was reported. Overall presence or absence of norovirus showed a significant association with illness complaints. The batch with the highest norovirus RNA levels also resulted in the highest rate of reported illness, suggesting a linkage between virus RNA levels and health risks.

Attachment of Noroviruses to Stainless Steel and Their Inactivation, Using Household Disinfectants

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Significance: Strategies for decontaminating surfaces harboring norovirus should be improved, thereby reducing the incidence of illness caused by these pathogens in the food sector and domestic environments.

The impact of pH and relative humidity on the attachment of norovirus (NoV) to fomites and the effectiveness of different household disinfectants on NoV attached to fomites were evaluated. Plaque assay and/or real-time reverse transcription (RTRT) PCR assay were used to determine the amount of murine and human NoV attached to stainless steel disks. An enzymatic pretreatment was used for both human and murine NoV before the RTRT-PCR assay to avoid detection of RNA associated with inactivated virus. For both murine and human NoV, maximum attachment was obtained after a contact time of 10 min. Attachment of NoV to stainless steel does not appear to be affected by pH, although murine NoV was less attached (<2 log units) at pH 9 and at low relative humidity (25%) than was human NoV (3 log units). Sodium hypochlorite (3%) was the most effective disinfectant. Murine NoV was more sensitive than human NoV to disinfectants by approximately 1-2 log units.

Mycotoxins

Four-Year Surveillance for Ochratoxin A and Fumonisin in Retail Foods in Japan

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Significance: Ochratoxin A and fumonisins were detected in several food products in Japan; however, their concentrations were relatively low.

Foods from Japanese retail shops were examined for contamination with ochratoxin A (OTA) (1,358 samples of 27 different products) and fumonisins B₁, B₂, and B₃ (831 samples of 16 different products). The limits of quantification ranged from 0.01-0.5 µg/kg for OTA and 2-10 µg/kg for the fumonisins. OTA was detected in amounts higher than limits of quantification in wheat flour, pasta, oatmeal, rye, buckwheat flour and dried buckwheat noodles, raisins, wine, beer, coffee beans and coffee products, chocolate, cocoa, and coriander. OTA was found in >90% of the samples of instant coffee and cocoa; the highest concentration of OTA, 12.5 µg/kg, was detected in raisins. The concentration of OTA in oatmeal, rye, raisins, wine, and roasted coffee beans varied remarkably from year to year. Fumonisin were detected in frozen and canned corn, popcorn grain, corn grits, cornflakes, corn soups, corn snacks, beer, soybeans, millet, and asparagus. The highest concentrations of fumonisins B₁, B₂, and B₃ were detected in corn grits (1,670, 597, and 281 µg/kg, respectively).

Bisphenol A

Determination of Bisphenol A in U.S. Infant Formulas: Updated Methods and Concentrations

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Significance: Bisphenol A (BPA) concentrations in liquid formula ($0.48\text{--}11\text{ ng g}^{-1}$) were consistent with previous studies, and BPA was detected in only 1 of 14 powder formula products analyzed.

An updated survey of U.S. infant formula was conducted to determine the concentrations of bisphenol A (BPA) to accurately assess BPA concentrations across the infant formula market and to determine if geographic location or can age influences BPA concentrations. A method was developed to measure BPA in formula utilizing isotope dilution, solid-phase extraction, and liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). The method was tested and found to be reproducible (10% relative standard deviation), reliable ($47 \pm 1\%$ recovery), and sensitive (0.15 ng g^{-1} method detection limit). Over 160 analyses were conducted using 104 formula containers representing 36 products. Samples from U.S. east and west coast markets demonstrated no significant difference, and concentrations in older cans were not higher.

Infant formula

Role of Radicals in the Lipid Peroxidation Products of Commercial Infant Milk Formula

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Significance: The introduction of formate anion diminishes the peroxide amounts initially present in infant milk formulas as well as those induced by radiation-induced oxidation.

Four commercial brands of infant milk formula were investigated, and the amount of shelf lipid peroxidation was determined by measuring malondialdehyde (MDA) or thiobarbituric acid reactive substance (TBARS) levels, which are the end products of lipid peroxidation. TBARS level, indicated by MDA concentration, was higher in the vegetarian brands. The vegetarian brands are more prone to oxidation than dairy brands, as they contain more unsaturated fats.