

Salmonella

Detection of Viable *Salmonella* in Lettuce by Propidium Monoazide Real-Time PCR

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Significance: The propidium monoazide real-time PCR assay provides an alternative to real-time PCR assay for accurate detection of *Salmonella* in food.

This study investigated the stability of DNA of dead *Salmonella* cells in lettuce and developed an approach to detect viable *Salmonella* in lettuce. *Salmonella*-free lettuce was inoculated with heat-killed *Salmonella* Typhimurium cells and stored at 4°C. Bacterial DNA extracted from the sample was amplified by real-time PCR targeting the *invA* gene. Results indicated that DNA from the dead cells remained stable in lettuce for at least 8 d. To overcome this limitation, propidium monoazide (PMA), a dye that can selectively penetrate dead bacterial cells and cross-link their DNA upon light exposure, was combined with real-time PCR. Lettuce samples inoculated with different levels of dead or viable *S. Typhimurium* cells were treated or untreated with PMA before DNA extraction. Real-time PCR suggests that PMA treatment effectively prevented PCR amplification from as high as 10⁸ CFU/g dead *S. Typhimurium* cells in lettuce. The PMA real-time PCR assay could detect viable *Salmonella* at as low as 10² CFU/mL in pure culture and 10³ CFU/g in lettuce. With 12-h enrichment, *S. Typhimurium* of 10¹ CFU/g in lettuce was detectable.

E. Coli

Quantitative Assessment of the Microbial Risk of Leafy Greens from Farm to Consumption: Preliminary Framework, Data, and Risk Estimates

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Significance: This risk assessment model represents a preliminary framework that identifies available data and provides initial risk estimates for pathogenic *E. coli* in leafy greens.

This project was undertaken to relate what is known about the behavior of *Escherichia coli* O157:H7 under laboratory conditions and integrate this information to what is known regarding the 2006 *E. coli* O157:H7 spinach outbreak in the context of a quantitative microbial risk assessment. The risk model explicitly assumes that all

contamination arises from exposure in the field. Extracted data, models, and user inputs were entered into an Excel spreadsheet, and the modeling software @RISK was used to perform Monte Carlo simulations. The model predicts that cut leafy greens that are temperature abused will support the growth of *E. coli* O157:H7, and populations of the organism may increase by as much as 1 log CFU/day under optimal temperature conditions. When the risk model used a starting level of -1 log CFU/g, with 0.1% of incoming servings contaminated, the predicted numbers of cells/serving were within the range of best available estimates of pathogen levels during the outbreak. The model predicts that levels in the field of -1 log CFU/g and 0.1% prevalence could have resulted in an outbreak approximately the size of the 2006 *E. coli* O157:H7 outbreak.

Effect of Repeated Irrigation with Water Containing Varying Levels of Total Organic Carbon on the Persistence of *Escherichia coli* O157:H7 on Baby Spinach

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Significance: Under growth chamber conditions, *E. coli* O157:H7 populations in irrigation water that complies with the Leafy Greens Marketing Agreement standards will not persist for more than 24 h when applied onto foliar surfaces of spinach plants.

The California lettuce and leafy greens industry has adopted the Leafy Greens Marketing Agreement (LGMA), which allows for 126 most-probable-number (MPN) *Escherichia coli* per 100 ml in irrigation water. Repeat irrigation of baby spinach plants with water containing *E. coli* O157:H7 and different levels of total organic carbon (TOC) was used to determine the epiphytic survival of *E. coli* O157:H7. Three irrigation treatments (0 ppm of TOC, 12 or 15 ppm of TOC, and 120 or 150 ppm of TOC) were prepared with bovine manure containing *E. coli* O157:H7 at either low (0-1 log CFU/100 ml) or high (5-6 log CFU/100 ml) populations, and sprayed onto baby spinach plants in growth chambers. MPN and direct plating techniques were used to determine the *E. coli* O157:H7 populations on the aerial plant tissue. Plants irrigated with high *E. coli* O157:H7 populations showed a 3-log reduction within the first 24 h. Low levels of *E. coli* O157:H7 were observed for ≤ 16 days on all TOC treatments, ranging from 76.4 MPN/plant (day 1) to 0.40 MPN/plant (day 16). No viable cells were detected on spinach tissue 24 h after irrigation with water containing fewer than 126 CFU/100 ml *E. coli* O157:H7.

Effect of Modified Atmosphere Packaging on the Persistence and Expression of Virulence Factors of *Escherichia coli* O157:H7 on Shredded Iceberg Lettuce

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Significance: Storage under near-ambient air atmospheric conditions can promote higher expression levels of *E. coli* O157 virulence factors on lettuce, and could affect the severity of *E. coli* O157:H7 infections associated with leafy greens.

Packaging conditions and abusive storage temperatures of contaminated lettuce were evaluated for their effect on the potential virulence of *Escherichia coli* O157:H7. Shredded lettuce was inoculated with 5.58 and 3.98 log CFU *E. coli* O157:H7/g and stored at 4 and 15°C, respectively, for ≤10 days. Lettuce was packaged under treatment A (in a gas-permeable film with N₂), treatment B (in a gas-permeable film with microperforations), and treatment C (in a gas-impermeable film). RNA was extracted from packaged lettuce for analysis of expression of virulence factor genes *stx*₂, *eae*, *ehxA*, *iha*, and *rfbE*. *E. coli* O157:H7 populations on lettuce at 4°C under all treatments decreased, but most considerably under treatment B over 10 days. At 15°C, *E. coli* O157:H7 populations increased by at least 2.76 log CFU/g under all treatments. At 15°C, expression of *eae* and *iha* was significantly greater under treatment B than it was under treatments A and C on day 3. Similarly, treatment B promoted significantly higher expression of *stx*₂, *eae*, *ehxA*, and *rfbE* genes on day 10, compared with treatments A and C at 15°C.

Food Allergy

Early Complementary Feeding and Risk of Food Sensitization in a Birth Cohort

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Link to full text: [http://www.jacionline.org/article/S0091-6749\(11\)00343-5/fulltext](http://www.jacionline.org/article/S0091-6749(11)00343-5/fulltext)

Significance: Complementary food introduced <4 months was associated with a reduced risk of peanut (and perhaps egg) sensitization by age 2 to 3 years, but only for children with a parental history of asthma or allergy.

The relationship between introduction of complementary food <4 months and IgE to egg, milk, and peanut allergen at 2 years were explored. Mothers of infants aged 1, 6, and 12 months were interviewed about feeding practices. Blood samples were collected at age 2 to 3 years to assess sensitization (IgE ≥0.35 IU/mL) to egg, milk, or peanut. Infant exposure to complementary food <4 months was reported by 39.7% of mothers. IgE ≥0.35 IU/mL for egg, milk, or peanut allergen at age 2 years was observed in 23.9% (95% CI, 20.5%- 27.6%), 30.6% (26.9%-34.5%), and 11.4% (8.9%-14.3%) of children, respectively. The association between early feeding and sensitization was modified by parental history of asthma or allergy. In multivariate analysis, early feeding reduced the risk of peanut sensitization among children with a parental history (adjusted OR=0.2 [95% CI, 0.1-0.7]; *P*=.007). The relationship also became significant for egg when a cutoff for IgE of ≥0.70 IU/mL was used (adjusted OR=0.5 [95% CI, 0.3-0.9]; *P*=.022).

Ara h 1-reactive T Cells in Individuals with Peanut Allergy

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Link to full text: [http://www.jacionline.org/article/S0091-6749\(11\)00353-8/fulltext](http://www.jacionline.org/article/S0091-6749(11)00353-8/fulltext)

Significance: Class II tetramers can be readily used to detect Ara h 1–reactive T cells in the peripheral blood of subjects with peanut allergy without *in vitro* expansion.

This study identified, characterized, and tracked Ara h 1–reactive cells in subjects with peanut allergy by using Ara h 1–specific class II tetramers. Tetramer-guided epitope mapping was used to identify the antigenic peptides within the peanut allergen Ara h 1. Subsequently, HLA class II/Ara h 1–specific tetramers were used to determine the frequency and phenotype of Ara h 1–reactive T cells in subjects with peanut allergy. Ara h 1–specific CD4+ T cells were detected in all of the subjects with peanut allergy tested. Ara h 1–reactive T cells in subjects with allergy expressed CCR4, but did not express CRTH2. The percentage of Ara h 1–reactive cells that expressed the β 7 integrin was low compared with total CD4+ T cells. Ara h 1–reactive cells that secreted IFN- γ , IL-4, IL-5, IL-10, and IL-17 were detected.

Fruit and Vegetable Consumption in Relation to Allergy: Disease-Related Modification of Consumption?

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Significance: An inverse association between fruit intake and allergic disease in children was confirmed.

This study investigated the association between current fruit or vegetable intake and allergic disease in 2447 8-year-old Swedish children, and evaluated the potential effect of disease-related modification of consumption. An inverse relation was observed between total fruit consumption and rhinitis (OR, highest vs. lowest quartile, 0.62; 95% CI, 0.45-0.86; *P* for trend, .002), whereas no association was observed for total vegetable intake. In analyses of individual foods, intake of apples/pears and carrots was inversely associated with rhinitis, asthma, and atopic sensitization. Fifty percent of the children with rhinitis were sensitized against birch pollen, which may cross-react with apples and carrots. After exclusion of children who reported food-related allergic symptoms, most of the observed inverse associations moved toward the null and became nonsignificant.

Foodborne Pathogens

Prevalence of *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli* Virulence Factors in Bulk Tank Milk and In-Line Filters from U.S. Dairies

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Significance: Significant contamination of bulk tank milk by zoonotic bacterial pathogens and that the consumption of raw milk and raw milk products presents a health risk.

Bulk tank milk (BTM) samples (536) and in-line milk filters (519) collected from dairy farms across the U.S. during

the National Animal Health Monitoring System's Dairy 2007 study were analyzed by real-time PCR for the presence of *Salmonella enterica* and pathogenic forms of *Escherichia coli* and by culture techniques for the presence of *Listeria monocytogenes*. *S. enterica* was detected in samples from 28.1% of the dairy operations, primarily in milk filters. *Salmonella* was isolated from 36 of 75 PCR-positive BTM samples and 105 of 174 PCR-positive filter samples, and the isolates were serotyped. Cerro, Kentucky, Muenster, Anatum, and Newport were the most common serotypes. *L. monocytogenes* was isolated from 7.1% of the dairy operations, and the 1/2a complex was the most common serotype, followed by 1/2b and 4b (lineage 1). Shiga toxin genes were detected in enrichments from 15.2% of the BTM samples and from 51.0% of the filters by real-time PCR. In most cases, the cycle threshold values for the PCR indicated that toxigenic strains were not a major part of the enrichment populations.

Norovirus

Ozone Inactivation of Norovirus Surrogates on Fresh Produce

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Significance: Ozone is an alternative method to reduce viral contamination on the surface of fresh produce.

This study compared ozone inactivation of human norovirus surrogates (feline calicivirus [FCV] and murine norovirus [MNV]) on produce (green onions and lettuce) and in sterile water. Green onions and lettuce inoculated with FCV or MNV were treated with ozone (6.25 ppm) for 0.5- to 10-min time intervals. Infectivity was determined by 50% tissue culture infectious dose (TCID₅₀) and plaque assay for FCV and MNV, respectively. After 5 min of ozone treatment, >6 log TCID₅₀/ml of FCV was inactivated in water and 2-log TCID₅₀/ml on lettuce and green onions. MNV inoculated onto green onions and lettuce showed a >2-log reduction after 1 min of ozone treatment. The food matrix played the largest role in protection against ozone inactivation.

Year-Round Prevalence of Norovirus in the Environment of Catering Companies without a Recently Reported Outbreak of Gastroenteritis

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Link to full text: <http://aem.asm.org/cgi/content/full/77/9/2968>

Significance: Ozone is an alternative method to reduce viral contamination on the surface of fresh produce.

In a year-round prevalence study, the prevalence of norovirus (NoV) in catering companies without recently reported outbreaks of gastroenteritis was investigated and compared to the observed prevalence in catering companies with recently reported outbreaks. Swab samples were collected from surfaces in the kitchens and (staff) bathrooms in 832

randomly chosen companies and analyzed for the presence of NoV RNA. In total, 42 (1.7%) out of 2,496 environmental swabs from 35 (4.2%) catering companies tested positive. In contrast, NoV was detected in 147 (39.7%) of the 370 samples for 44 (61.1%) of the 72 establishments associated with outbreaks of gastroenteritis. NoV-positive swabs were more frequently found in winter, in specific types of companies (elderly homes and lunchrooms), and in establishments with separate bathrooms for staff. Sequence analysis showed that environmental strains were interspersed with strains found in outbreaks of illness in humans.

Inactivation of a Human Norovirus Surrogate, Human Norovirus Virus-Like Particles, and Vesicular Stomatitis Virus by Gamma Irradiation

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Applied and Environmental Microbiology, Vol. 77, No. 10; pp. 3507-3517, 2011

Link to full text: <http://aem.asm.org/cgi/content/full/77/10/3507>

Significance: Viruses are much more resistant to irradiation than bacterial pathogens.

A systematic study on the inactivation of a human norovirus surrogate (murine norovirus 1 [MNV-1]), human norovirus virus-like particles (VLPs), and vesicular stomatitis virus (VSV) by gamma irradiation was performed. The researchers demonstrated that MNV-1 and human norovirus VLPs were resistant to gamma irradiation. For MNV-1, only a 1.7- to 2.4-log virus reduction in fresh produce at the dose of 5.6 kGy was observed. However, VSV was more susceptible to gamma irradiation, and a 3.3-log virus reduction at a dose of 5.6 kGy in Dulbecco's modified Eagle medium (DMEM) was achieved. They further demonstrated that gamma irradiation disrupted virion structure and degraded viral proteins and genomic RNA, which resulted in virus inactivation. Using human norovirus VLPs as a model, the first evidence that the capsid of human norovirus has stability similar to that of MNV-1 after exposure to gamma irradiation is provided.