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North America

Food Safety Briefs

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E. coli

Occurrence of Coliform and Escherichia coli Contamination and Absence of Escherichia coli O157:H7 on Romaine Lettuce from Retail Stores in the Upper Midwest

J.D. Greve, M.S. Zietlow, K.M. Miller, J.L.E. Ellingson

Journal of Food Protection, Vol. 78, No. 9; pp. 1729–1732, 2015.

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Link to full text: [Click here](#)

Significance: There was an absence of E. coli O157:H7 and low levels of contamination with coliforms and other E. coli strains of whole romaine lettuce heads.

A total of 720 whole, romaine lettuce heads were purchased from retail locations in the Upper Midwest and assessed for coliform and Escherichia coli contamination and for the presence of E. coli O157:H7. During a 16-month period (August 2010 through December 2011), coliform and E. coli counts were enumerated on Petrifilm, and the presence of E. coli O157:H7 and the virulence gene eae was evaluated by real-time PCR (qPCR). Over half (400 of 720) of the lettuce samples were processed with an immunomagnetic separation step before the qPCR assay. All retail lettuce samples were negative for E. coli O157:H7 when tested with the R.A.P.I.D. LT qPCR targeting a region of the O-antigen, and only two (0.28%) were positive for the eae gene when tested with LightCycler qPCR. On Petrifilm, coliform counts of most lettuce samples (96.4%) were between <101 and 103 CFU/g, and E. coli counts for nearly all lettuce samples (98.2%) were <101 CFU/g.

Enumeration of Escherichia coli O157 in Outbreak-Associated Gouda Cheese Made with Raw Milk

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Significance: E. coli O157 could not replicate during storage at 4°C in the products tested but was capable of survival following aging and prolonged storage.

The enumerative analysis for Escherichia coli O157 in two raw milk Gouda cheese products (A and B) implicated in an outbreak of 29 cases of E. coli O157:H7 illness that occurred across Canada in 2013 are reported. Samples were enumerated for E. coli O157 by most probable number (MPN) over a period of 30 to 60 days after the end of the outbreak. Samples (55.55 g) of product A (n=14) were analyzed at 146 to 180 days postproduction. E. coli O157 was isolated from six samples at 19.9 to 44.6 MPN/kg. The E. coli O157 concentration of product A estimated from the results of all 14 samples was 9.5 MPN/kg. Samples (55.55 g) of product B (n=20) were analyzed at 133 to 149 days postproduction. E. coli O157 was isolated from four

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samples at 19.9 MPN/kg. The *E. coli* O157 concentration of product B estimated from the results of all 20 samples was 3.7 MPN/kg. Analysis of a 305-g sample of product A (n=1) stored at 4°C until 306 days postproduction revealed that the *E. coli* O157 concentration had declined to 3.6 MPN/kg. *E. coli* O157 could not be isolated from 555-g samples of product B (n=5) after 280 days postproduction.

Salmonella

Evaluation of Rapid Molecular Detection Assays for Salmonella in Challenging Food Matrices at Low Inoculation Levels and Using Difficult-to-Detect Strains

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Significance: Rapid assays may have high false-negative rates when performance is evaluated under challenging conditions, including low-moisture matrices, strains that are difficult to detect, injured cells, and low inoculum levels.



This study was conducted to evaluate a secondary end user validation of four AOAC-validated commercial rapid detection assays (an isothermal nucleic acid amplification, an immunoassay, and two PCR-based assays) for their ability to detect *Salmonella* in two challenging matrices (dry pet food and dark chocolate). Inclusivity was evaluated with 68 diverse *Salmonella* strains at low population levels representing the limit of detection (LOD) for each assay. One assay detected all strains at the LOD, two assays detected multiple strains only at 10 times the LOD, and the fourth assay failed to detect two strains (*Salmonella bongori* and *S. enterica* subsp. *houtenae*) even at 1,000 times the LOD; this assay was not further evaluated. The three remaining assays were subsequently evaluated for their ability to detect five selected *Salmonella* strains in food samples contaminated at fractional levels. Unpaired comparisons revealed no significant difference between the results for each given assay and the results obtained with the reference assay. However, analysis of paired culture-confirmed results revealed assay false-negative rates of 4 to 26% for dry pet food and 12 to 16% for dark chocolate.

Application of Water-Assisted Pulsed Light Treatment to Decontaminate Raspberries and Blueberries From Salmonella

Y. Huang, R. Sido, R. Huang, H. Chen

International Journal of Food Microbiology, Vol. 208, 2 September 2015; pp. 43–50, 2015.

DOI: 10.1016/j.ijfoodmicro.2015.05.016

Link to full text: [Click here](#)

Significance: The combination of water-assisted pulsed light and hydrogen peroxide resulted in significantly higher reduction of *Salmonella* on berries, which provides a novel intervention for processing of small berries intended for fresh-cut and frozen berry products.

A small scaled-up water-assisted pulsed light (WPL) system was developed and evaluated wherein berries were washed in a flume washer while being irradiated by pulsed light (PL). Hydrogen peroxide (H₂O₂) was used in combination with PL as an advanced oxidation process and chlorine wash was used as a control. The effects of organic load, water turbidity, berry type and PL energy output on the

inactivation of *Salmonella* using the WPL system were investigated. The combination of WPL and 1% H_2O_2 (WPL- H_2O_2) was the most effective treatment which reduced *Salmonella* on raspberries and blueberries by 4.0 and > 5.6 log CFU/g, respectively, in clear water. When high organic load and SiO_2 , as a soil simulator, were added in wash water, the free chlorine level in chlorinated water decreased significantly. Even in the presence of high organic load and water turbidity, no viable bacterial cells were recovered from the wash water, which showed that WPL- H_2O_2 could effectively prevent the risk of cross-contamination during treatment. Taken together, 1-min WPL treatment without H_2O_2 could provide a chemical free alternative to chlorine washing with similar and significantly higher bactericidal efficacy.

Foodborne Pathogens

Role of Brushes and Peelers in Removal of *Escherichia coli* O157:H7 and *Salmonella* from Produce in Domestic Kitchens

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Link to full text: [Click here](#)

Significance: Risk of cross-contamination from contaminated utensils to uncontaminated produce may be limited.

This study monitored the fate of *Escherichia coli* O157:H7 and *Salmonella* on produce (cantaloupe, honeydew melon, carrots, and celery) that was subjected to brushing or peeling using common kitchen utensils. Removal of similar levels of *Salmonella* from carrots was accomplished by peeling and by brushing, but significantly greater removal of *E. coli* O157:H7 from carrots was accomplished by peeling than by brushing under running water. Brushing removed significantly fewer pathogens from contaminated cantaloupes than from other produce items. A Sparta polyester brush was less effective than a scouring pad for removing *Salmonella* from carrots. In all cases, brushing and peeling failed to eliminate the pathogens from the produce items, which may be the result of contamination of the utensil during use. High incidences of contamination (77 to 92%) were found among peelers used on carrots or celery, the Sparta brush used on carrots, and the scouring pad used on carrots and cantaloupe. Of the utensils investigated, the nylon brush had the lowest incidence of pathogen transference from contaminated produce (0 to 12%). Transfer of pathogens from a potentially contaminated Sparta brush or peeler to uncontaminated carrots did not occur or occurred only on the first of seven carrots processed with the utensil.

Microbiological Testing Results of Boneless and Ground Beef Purchased for the National School Lunch Program, 2011 to 2014

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Significance: Beef produced for the Agricultural Marketing Service National School Lunch Program is done under an adequate food safety system as indicated by the low percentage of lots that were pathogen positive or exceeded critical limits for indicator organisms.



The Agricultural Marketing Service (AMS) purchases boneless and ground beef for distribution to recipients through federal nutrition assistance programs, including the National School Lunch Program, which represents 93% of the overall volume. From July 2011 through June 2014, 537,478,212 lbs. (ca. 243,795,996 kg) of boneless beef and 428,130,984 lbs. (ca. 194,196,932 kg) of ground beef were produced for these programs. Of the 230,359 boneless beef samples collected over this period, 82 (0.04%) were positive for *E. coli* O157:H7, 924 (0.40%) were positive for *Salmonella*, 222 (0.10%) exceeded the critical limit for standard plate count organisms (SPCs), 69 (0.03%) exceeded the critical limit for *E. coli*, and 123 (0.05%) exceeded the critical limit for coliforms. Of the 46,527 ground beef samples collected over this period, 30 (0.06%) were positive for *E. coli* O157:H7, 360 (0.77%) were positive for *Salmonella*, 20 (0.04%) exceeded the critical limit for SPCs, 22 (0.05%) exceeded the critical limit for *E. coli*, and 17 (0.04%) exceeded the critical limit for coliforms.

Microbiological Analysis of Pre-Packed Sweet Basil (*Ocimum Basilicum*) and Coriander (*Coriandrum Sativum*) Leaves for the Presence of *Salmonella* Spp. and Shiga Toxin-Producing *E. Coli*

Stefanie Delbeke, Siele Ceuppens, Liesbeth Jacxsens, Mieke Uyttendaele
International Journal of Food Microbiology, Vol. 208, 2 September 2015; pp. 4311–4318, 2015.

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Link to full text: [Click here](#)



Significance: Fresh leafy herbs like basil and coriander, sourced from different cultivation regions, may contain enteric pathogens and potentially pose a risk for human health.

Fresh pre-packed basil and coriander leaves were investigated for the presence of *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), generic *E. coli* and coliforms. Multiplex PCR followed by further culture confirmation was used for the detection of *Salmonella* spp. and STEC, whereas the Petrifilm Select *E. coli* and VRBL-agar were used, respectively, for the enumeration of *E. coli* and coliforms. *Salmonella* was detected in 10 out of 592 samples (25 g) (1.7%; 5 from basil and 5 from coriander). The presence of STEC was suspected in 11 out of 592 samples (25 g) (1.9%; 3 basil and 8 coriander), due to the detection of *stx* and *eae* genes. No STEC was isolated by culture techniques, but in three samples a serotype (O26, O103 or O111) with its most likely associated *eae*-variant (β or θ) was detected by PCR. Generic *E. coli* was enumerated in 108 out of 592 samples, whereby 55, 32 and 13 samples respectively between 10–100, 100–1000 and 1000–10,000 CFU/g and 8 samples exceeding 10,000 CFU/g. Coliforms were enumerated in all herb samples at variable levels ranging from 1.6 to 7.5 log CFU/g. Further statistics indicate that the *E. coli* class was significantly correlated with the presence of *Salmonella* ($p < 0.001$) or STEC ($p = 0.019$), while coliform counts were significant correlated with *Salmonella* ($p < 0.001$), but not with STEC ($p = 0.405$). Generic *E. coli* class is a better indicator for the presence of enteric pathogens than coliforms on fresh herbs.

Listeria

Modeling the Effect of Storage Temperatures on the Growth of *Listeria monocytogenes* on Ready-to-Eat Ham and Sausage

K. Luo, S-S. Hong, D-H. Oh

Journal of Food Protection, Vol. 78, No. 9; pp. 1675–1681, 2015.

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Link to full text: [Click here](#)

Significance: Standardized predictive models can be used to improve the food safety of ready-to-eat meat products.

The growth kinetics of *Listeria monocytogenes* on ready-to-eat ham and sausage was modeled at different temperatures (4 to 35°C). The observed data fitted well with four primary models (Baranyi, modified Gompertz, logistic, and Huang) with high coefficients of determination ($R^2 > 0.98$) at all measured temperatures. After the mean square error, bias factors, and accuracy factors were obtained in all models, the square root and the natural logarithm model were employed to describe the relation between temperature and specific growth rate (SGR) and lag time (LT) derived from the primary models. These models were validated against the independent data observed from additional experiments using the acceptable prediction zone method and the proportion of the standard error of prediction. All secondary models based on each of the four primary models were acceptable to describe the growth of the pathogen in the two samples. The validation results indicate that the optimal primary model for estimating the SGR was the Baranyi model, and the optimal primary model for estimating LT was the logistic model in ready-to-eat (RTE) ham. The Baranyi model was also the optimal model to estimate the SGR and LT in RTE sausage.

Spatial and Temporal Factors Associated with an Increased Prevalence of *Listeria monocytogenes* in Spinach Fields in New York State

D. Weller, M. Wiedmann, L.K. Strawn

Applied and Environmental Microbiology, Vol. 81, No. 17; pp. 6059–6069, 2015.

DOI: 10.1128/AEM.01286-15

Link to full text: [Click here](#)

Significance: Small changes in management practices (e.g., not irrigating fields before harvest) may therefore reduce the risk of *Listeria monocytogenes* contamination of fresh produce.

This study was performed to quantify various spatial and temporal factors on the risk of produce contamination following rain and irrigation events, and to determine the impact of these events on the detection frequency and diversity of *Listeria* species (including *L. monocytogenes*) and *L. monocytogenes* in produce fields. Two spinach fields, with high and low predicted risks of *L. monocytogenes* isolation, were sampled 24, 48, 72, and 144 to 192 h following irrigation and rain events. Factors were evaluated for their association with *Listeria* species and *L. monocytogenes* isolation by using generalized linear mixed models (GLMMs). In total, 1,492 (1,092 soil, 334 leaf, 14 fecal, and 52 water) samples were collected. According to the GLMM, the likelihood of *Listeria* species and *L. monocytogenes* isolation from soil samples was highest during the 24 h immediately following an event ORs of 7.7 and 25, respectively). Additionally, *Listeria* species and *L. monocytogenes* isolates associated with irrigation events showed significantly lower sigB allele type diversity than did isolates associated with precipitation events ($P < 0.001$), suggesting that irrigation water may be a point source of *L. monocytogenes* contamination.



Mycotoxins

Biomonitoring of Concurrent Mycotoxin Exposure Among Adults in Sweden Through Urinary Multi-Biomarker Analysis

S. Wallin, L. Gambacorta, N. Kotova, E.W. Lemming, C. Nälsén, M. Solfrizzo, et al.

Food and Chemical Toxicology, Vol. 83, September 2015; pp. 133–139, 2015.

DOI: 10.1016/j.fct.2015.05.023

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Significance: This is the first study to investigate concurrent mycotoxin exposure and urinary levels of fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), nivalenol (NIV), ochratoxin A (OTA), zearalenone (ZEA), α -zearalenol (α -ZOL), β -zearalenol (β -ZOL) and de-epoxydeoxynivalenol (DOM-1) among adults.

This study examined concurrent exposure to mycotoxins through urinary multi-biomarker analysis, as well as its possible associations with the diet. Urinary samples from 252 adults were collected together with a 4-day diet record. Concurrent mycotoxin exposure was studied using a multi-biomarker LC-MS/MS method. The results revealed that exposure to mycotoxins is common and concurrent exposure to more than one toxin was found in 69% of the study population. However, when comparing the number of toxins detected with the reported consumption data, it was difficult to distinguish food patterns that would indicate an increased risk of exposure to many mycotoxins simultaneously.



Bisphenols

Monitoring of Bisphenols in Canned Tuna From Italian Markets

M. Fattore, G. Russo, F. Barbato, L. Grumetto, S. Albrizio

Food and Chemical Toxicology, Vol. 83, September 2015; pp. 68–75, 2015.

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Link to full text: [Click here](#)

Significance: On the basis of measured concentrations and general daily ingestion rate of canned tuna fish, the probable daily intake of BPA was calculated.

A method for the quali-quantitative analysis of Bisphenol A (BPA), Bisphenol B (BPB), Bisphenol A Diglycidyl Ether (BADGE), and Bisphenol F Diglycidyl Ether (BFDGE), by liquid chromatography with fluorescence detection (LC-FD), was performed and validated for their determination in 33 samples of tuna fish, canned in either oil or aqueous medium. Tuna and the correspondent preservation medium were analyzed separately. Detected levels of bisphenols ranged from 19.1 to 187.0 ng/g in tuna matrix and from 6.3 to 66.9 ng/mL in oil medium. No bisphenols were found in aqueous medium. At least one of the analytes was found in 83% of the tuna samples in oil medium, whereas tuna samples in aqueous medium showed BPA alone in 67% of samples. 21% of the oil medium samples resulted positive for at least one bisphenol.

Estimation of *In Vivo* and *In Vitro* Exposure to Bisphenol A as Food Contaminant

N. Milić, D. Četojević-Simin, M. Milanović, J. Sudji, N. Milošević, N. Čurić, et al.

Food and Chemical Toxicology, Vol. 83, September 2015; pp. 268–274, 2015.

DOI: 10.1016/j.fct.2015.07.003

Link to full text: [Click here](#)

Significance: A higher exposure to bisphenol A could contribute to weight problems in women.

This cross-sectional study examined the occurrence of bisphenol A (BPA) in the morning spot urine taken from 145 female volunteers of various ages. Total urine BPA concentration was detected in 38.6% samples in the 0.92–70.96 µg/g Cr range. The majority of BPA + women belonged to the 25 + BMI group (54.5% were overweight and 43.4% were obese women). Occurrence of BPA in the urine samples was higher at 40 + ages. The maximum BPA concentration of 70.96 µg/g Cr was detected in the urine sample of an obese woman. It is known that BPA is highly toxic *in vitro*. In this study BPA impaired significantly the growth of all investigated cell lines (i.e., the EC₅₀ values were reached at very low concentrations, in the range from 3.24 to 34.85 µg/mL). The absence of BPA *in vitro* selective toxicity studies indicates to its general toxic mode of action and raises awareness of the health risks associated with its ubiquitous presence in the environment.

Heavy Metals

Content of Cadmium and Lead in Vegetables and Fruits Grown in the Campania Region of Italy

M. Esposito, G. Picazio, P. Serpe, S. Lambiase, P. Cerino

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Significance: Samples of tomatoes exceeded the maximum level of cadmium, and valerian contained an excess of lead.

To assess the levels of contamination by lead and cadmium, the only metals subject to European Union legislation, a system of monitoring of plant foods in the whole territory of the region has been promoted, with the goal of certifying productions and consumer protection. In fact, products that comply with European Union standards are assigned a Quick Response Code, which guarantees the traceability of the product (manufacturer and location). The code also ensures the safety of the product, as it allows the consumer to see the results of the analysis performed on the specific chain of production. The content of lead and cadmium was determined in 750 vegetable samples by using the atomic absorption spectrophotometry after microwave mineralization. These levels were below the maximum limits in all but three samples.

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