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Vol. 66 **2003** **Supplement A**

Table of Contents

Developing Scientist Competitors

Allan, John, University of Georgia (T53)

Annamalai, Thirunavukkarasu, University of Connecticut (P036)

Armstrong, Ken, Uniersity of Tennessee-Knoxville (P117)

Ashton, Laura, Colorado State University (P023)

Bieker, Jill, Kansas State University (P205)

Bisha, Bledar, Iowa State University (P191)

Brown, Kristen, University of Guelph (P043)

Chapman, Benjamin, University of Guelph (T18)

Crozier-Dodson, Beth, Kansas State University (T03)

Drake, Jayne, University of Wales Institute-Cardiff (P196)

Eimerman, Patrick, University of Wisconsin-Madison (P138)

Evans, Matthew, North Carolina State University (P049)

Favrin, Stacy, University of Guelph (P047)

Felkey, Kelly, University of Florida (P212)

Fluckey, Wade, Texas Tech University (T46)

Folsom, James, University of Gergia (P016)

Gomes, Liz, University of Guelph (T23)

Groves, Errol, University of Vermont (P078)

Hartman, Angela, Virginia Tech (P246)

Hayburn, Gordon, University of Wales Institute-Cardiff (P134)

Hereford, Megan, Virginia Tech (P244)

Johnson, Clint, Iowa State University (P033)

Kashi, Yechezkel, Technion (P015)

Kenney, Stephen, University of Georgia (P054)

Keskinen, Lindsey, Michigan State University (P044)

Kim, Hye-Jung, Gyeongsang National University (P266)

Kleman, Lynette, North Carolina State University (T33)

Korsak, Nicolas, Liege University (P093)

Kottapalli, Balasubrahmanyam, North Dakota State University (P228)

Lamar, Kimberly, University of Tennessee-Knoxville (P141)

Lang, Megan, University of Georgia (P022)

Lappi, Victoria, Cornell University (T52)

Lee, Je-Myung, Gyeongsang National University (P199)

Lee, Kyung-Mi, Yonsei University (P202)

Ling, Valerie, University of Tennessee-Knoxville (P011)

Marek, Patrick, University of Connecticut (T10)

Mathiasen, Lisa, University of Guelph (T24)

Mohan Nair, Manoj Kumar, University of Connecticut (P136)

Moore, Ginny, University of Wales Institute-Cardiff (T14)

Ngutter, Crystal, University of Vermont (T11)

Paik, Sujin, Iowa State University (P185)

Palekar, Mangesh, Texas A&M University (P238)

Panicker, Gitika, University of Alabama-Birmingham (T04)

Pierre, Pascale, Michigan State University (P115)

Piotrowski, Christine, Virginia Tech (P247)

Ransom, Justin, Colorado State University (T48)

Rasanayagam, Vasuhi, NCFST, Illinois Institute of Technology (P099)

Rash, Vivian, University of Tennessee (P240)

Richards, Glenner, University of Georgia, CFS (P236)

Romero, Maria, Iowa State University (P124)

Sharma, Manan, University of Georgia (T45)

Shim, Won-Bo, Gyeongsang National University (P113)

Sloan, Kristin, University of Guelph (P009)

Smith, Katie, North Carolina State University (P125)

Smith, Sarah, Rutgers University (P256)

Solnik, Hadas, Technion (P232)

Stanley, Kimberly, University of Tennessee (P021)

Stevens, Kelly, North Carolina State University (P107)

Stopforth, Jarret, Colorado State University (P028)

Tetteh, Gloria, University of Georgia (P032)

Tipparaju, Sireesha, Illinois Institute of Technology (P139)

Vasudevan, Pradeep, University of Connecticut (P008)

Vorst, Keith, Michigan State University (T15)

Waddington, Lisa, Lakehead University (P094)

Wieckowicz, Alison, University of Wisconsin-River Falls (P066)

Yang, Zheng-You, Gyeongsang National University (P225)

Yoo, Mi-young, Kangwon National University (P254)

Yoon, Yohan, Colorado State University (P155)

Younts-Dahl, Spring, Texas Tech University (T13)

Yuk, Hyun-Gyun, Mississippi State University (P020)

Presenter List

P – Poster; S – Symposium; T – Technical

Allan, John, University of Georgia (T53)

Annamalai, Thirunavukkarasu, University of Connecticut (P036)

Annous, Bassam A., USDA-ARS-ERRC (T29)

Apostilides, Emmanoui, University of Massachusetts (P183)

Arbault, Patrice, Diffchamb SA (P060, P067, P111)

Arbogast, James W., GOJO Industries, Inc. (T16)

Armstrong, Ken A., University of Tennessee-Knoxville (P117)

Ashton, Laura V., Colorado State University (P023, P027, P037)

Baeumler, Andreas J., Texas A&M University (S22)

Bailey, Roger A., University of Wales Insitute-Cardiff (T25)

Bailey, Stan, USDA-ARS-RRC (P089, S11)

Bari, Latiful, National Food Research Institute (P267)

Barmpalia, Ioanna M., Colorado State University (P163)

Bayles, Darrell O., USDA-ARS (P074)

Ben Embarek, Peter K., WHO (S21)

Bernardo, Fernando M. A., CIISA-Faculdade Medicina Veterinária (P114)

Berrang, Mark E., USDA-ARS (P122)

Betts, Roy, Campden & Chorleywood Food Research Association (T37, S18)

Beuchat, Larry R., University of Georgia, CFS (P252, P257, S12, S19)

Bhaduri, Saumya, USDA-ARS-ERRC (P128)

Bhatnagar, Deepak, USDA-SRRC (S12)

Bidawid, Sabah, Health Canada (P031)

Bieker, Jill M., Kansas State University (P205)

Bisha, Bledar, Iowa State University (P165, P191)

Bolger, Michael, FDA-CFSAN (S17)

Boor, Kathryn J., Cornell University (S07)

Brassard, Julie, Canadian Food Inspection Agency (P233)

Brodsky, Michael H., Brodsky Consultants (S09)

Brown, Kristen, University of Guelph (P043)

Brul, Stanley, University of Amsterdam/ Unilever Research (S12, S14)

Buchanan, Robert L., FDA-CFSAN (S01)

Bullerman, Lloyd L., University of Nebraska-Lincoln (P188, S12)

Buncic, Sava, University of Bristol (P144)

Butner, Julie, Compass Group (S20)

Calci, Kevin R., FDA (P275)

Call, Jeffrey E., USDA-ARS-ERRC (P052)

Campos, Danilo T., Michigan State University (T61)

Carr, J. P., North Carolina A&T State University (P140)

Carter, Mark, Kraft Foods, Inc. (S07, S18)

Carter-Young, Heather L., RTI International (P168)

Carver, Charles, rtech laboratories (P072)

Casanova, Raiza N., Universidad Central de Venezuela (P119)

Casteel, Michael J., University of North Carolina-Chapel Hill (P097)

Cates, Sheryl C., RTI International (P160)

Cebula, Tom A., FDA (S22)

Chae, Joon-seok, Chonbuk National University (P280)

Chapman, Benjamin J., University of Guelph (T18)

Chen, Jinru, University of Georgia (P019, P025, P243)

Chen, Yuhuan, NFPA (S03)

Cloak, Orla, Strategic Diagnostics Inc. (P064)

Coger, Robert R., Kansas State University (P005)

Cohen, Larry, Kraft Foods (S11)

Cohn, Sheila, National Restaurant Association (S10)

Collins, Marina V, Virginia Tech (P287)

Contreras Romo, Paola Sabina, SC Universidad Veracruzana (P215)

Cooley, Michael B., USDA-ARS-WRRC (P269)

Cousin, Maribeth A., Purdue University (S12)

Croci, Luciana, Italian National Institute of Health (P090)

Cronin, Caroline E., University of Massachusetts (P273)

Crozier-Dodson, Beth A., Kansas State University (T03)

Cutter, Catherine N., Pennsylvania State University (P055, P281)

D'Souza, Doris H., North Carolina State University (T07)

Davidson, Dean, FDA-CFSAN (S20)

Davidson, P. Michael, University of Tennessee (S23)

Davidson, Valerie J., University of Guelph (P178, T63)

Davies, Rob H., Veterinary Laboratories Agency-Weybridge (T50)

De Medici, Dario, Italian National Institute of Health (P091)

Delaquis, Pascal J., Agriculture and Agri-Food Canada (T30)

DeLong, Stephanie M., CDC (T49)

DePaola, Andy, FDA (S08)

Desmarchelier, Patricia, Food Science Australia (P083)

Destro, Maria T., Universidade de Sâo Pâulo (P070, P272)

Dharmavaram, S.R.K., North Carolina A & T State University (P002)

DiGeronimo, Michael, Dover Brook Associates (S07)

Donnelly, Catherine W., University of Vermont (S01, S07)

Downing, Garvin, Ontario Ministry of Agriculture and Food (P286)

Doyle, Michael P., University of Georgia (S01, S18)

Drake, Jayne, University of Wales Institute-Cardiff (P196)

Duan, Jingyun, Oregon State University (P279)

Duff, Steven B., Covance Health Economics and Outcomes Services Inc. (P221)

Duffy, Karen S., National Diagnostics Centre (P076)

Duffy, Elizabeth A., Texas A & M University (P251)

Dykes, Gary A., Food Science Australia and University of Queensland (P270)

Edelson-Mammel, Sharon G., DHHS-FDA-CFSAN (P029)

Eifert, Joseph, Virginia Tech (P241, S04)

Eimerman, Patrick R., University of Wisconsin-Madison (P138)

El-Sukhon, Saeb Nazmi, Jordan University of Science and Technology (P034)

Ellis, Leanne, University of Wales Institute-Cardiff (T20)

Evans, Matthew R., North Carolina State University (P049)

Falci, Kenneth J., FDA-CFSAN (S10)

Favrin, Stacy J., University of Guelph (P047)

Felkey, Kelly D., University of Florida (P212)

Feng, Peter, FDA-CFSAN (S09)

Fenner-Crisp, Penelope A., ILSI Risk Science Institute (S17)

Fielding, Louise, University of Wales Institute-Cardiff (T17)

Fields, Patricia J., CDC (S09)

Fifadara, Nimita H., University of Georgia (P098)

Fleischman, Gregory J., FDA (P133)

Flick, Jr., George, Virginia Tech (S08)

Flores, Rolando A., USDA-ARS-ERRC (P152)

Fluckey, Wade M., Texas Tech University (T46)

Foley, Denise, Chapman University (P265)

Folsom, James P., University of Georgia (P016)

Foong, Sally C., Iowa State University (P051, P170)

Forney, David L., CDC (S20)

Frank, Joseph F., University of Georgia (P218)

Fratamico, Pina M., USDA-ARS-ERRC (P065, S09)

Frye, Cary, International Dairy Foods Association (S24)

Fu, Tong-Jen, FDA, NCFST (P268)

Garry, Eileen M., Advanced Instruments, Inc. (P103)

Gaulin, Colette, Ministry of Health, Province of Quebec (P137)

Gebler, Jill, Murray Goulburn Co-op Co. Ltd. (P087)

Gilbert, Carl D., University of Arkansas (P057)

Glass, Kathleen, University of Wisconsin-Madison (S02)

Goldman, David, USDA (S19)

Gomes, Liz V., University of Guelph (T23)

Gottfried, David S., Georgia Tech Research Institute (T12)

Gottlieb, Sami, CDC (S19)

Gravani, Robert B., Cornell University (T32)

Graves, Lewis M., CDC (P039)

Green, Laura R., RTI International (T41, T42)

Green, David, North Carolina State University (S08)

Greening, Gail E., Institute of Environmental Science and Research Ltd. (P274, P277)

Greer, Gordon, Agriculture and Agri-Food Canada, Lacombe Research Centre (P149)

Greig, Judy D., Health Canada (T66)

Griffith, Chris, University of Wales Institute (S05)

Griffiths, James C., Burdock Group (S17)

Grimm, Casey, USDA-ARS-SRRC (S21)

Groves, Errol, University of Vermont (P078)

Grower, Jennifer, Clemson University (S23)

Guentert, Ann M., Purdue University (P166)

Gurtler, Joshua B., University of Georgia (T01)

Gustilo, Khristine, N-terminus Research Laboratory (P146)

Guzewich, Jack, FDA-CFSAN (S03)

Han, Yingchang, Purdue University (T26)

Harris, Linda J., University of California-Davis (P234)

Hartman, Angela D., Virginia Tech (P246)

Hayburn, Gordon W., University of Wales Institute-Cardiff (P134, P198)

Hefle, Susan L., University of Nebraska (S10)

Hereford, Megan L., Virginia Tech (P244)

Herweyer, David, Wayne Chemical Co. (S02)

Hiett, Kelli, USDA-ARS-RRC (S15)

Himelbloom, Brian H., University of Alaska Fairbanks (P271)

Hinton, Jr., Arthur, USDA-RRC (P131)

Hirshfield, Irvin N., St. John's University (P259)

Hocking, Ailsa D., CSIRO-Food Science Australia (S12)

Holah, John T., Campden & Chorleywood Food Research Association (P214)

Holley, Richard A., University of Manitoba (P003, P017, P156)

Hollingsworth, Jill, Food Marketing Institute (S18)

Hollingworth, Robert, Michigan State University (S17)

Hoover, Dallas G., University of Delaware (S16)

Hopkins, Katie L., Central Public Health Laboratory (P231)

Horter, Barbara L., 3M Microbiology (P102)

Huang, Min, University of Tennessee-Knoxville (P248)

Huddle, Michelle, CDC (P042)

Hussein, Hussein S., University of Nevada-Reno (P014, P142)

Ibrahim, Salam A., North Carolina A&T State University (P104)

Islam, Mahbub U., University of Georgia (P262, P263)

Iturriaga, Montserrat H., Universidad Autónoma de Querétaro (P258)

Jackson, Felix R., University of Tennessee-Knoxville (P086)

Janes, Marlene E., Louisiana State University Agricultural Center (P056)

Jaykus, Lee-Ann, North Carolina State University (S03, S06)

Jean, Julie, North Carolina State University (T08)

Jechorek, Robert, rtech laboratories (P073)

Jessup, Amber, FDA-CFSAN (S11)

Johannessen, Gro S., National Veterinary Institute (T31)

Johnson, Michael G., University of Arkansas (P095)

Johnson, Eric A., University of Wisconsin-Madison (S23)

Johnson, Clint L., Iowa State University (P033)

Johnson, Jr., Daniel H., Clearview Medical Imaging (S24)

Juneja, Vijay K., USDA-ARS-ERRC (P130, P150)

Kashi, Yechezkel, Technion (P015)

Kasuga, Fumiko, National Institute of Health Sciences (T65)

Kaufman, Kathleen D., Cornell University (S07)

Kause, Janell R., USDA-FSIS-OPHS-RAD (T57)

Kawasaki, Susumu, National Food Research Institute (P105)

Kendall, David L., RTI International (P175)

Kennedy, Peter M., Quality Flow Inc. (S25)

Kennedy, James E., ABC Research Corporation (P190)

Kenney, Stephen J., University of Georgia (P054)

Keskinen, Lindsey A., Michigan State University (P044)

Kim, Jong-Gyu, Keimyung University (P229)

Kim, Hye-Jung, Gyeongsang National University (P266)

Kim, So Hyun, Seoul National University (P181, P187)

Kim, Myunghee, Korea Food Research Institute (P062)

Kim, Dong-Kyu, CJ Corporation (P059)

Kincaid, Jennifer A., CDC (P038)

Kleman, Lynette M., North Carolina State University (T33)

Kniel, Kalmia E., USDA-ARS (P227)

Knochel, Susanne, Royal Veterinary and Agricultural University (P035)

Kornacki, Jeffrey L., University of Georgia (T02)

Korsak, Nicolas, Liege University (P093)

Koseki, Shigenobu, Hokkaido University (P260)

Kottapalli, Balasubrahmanyam, North Dakota State University (P228)

Koutchma, Tatiana N., NCFST, IIT (T28)

Koutsoumanis, Konstantinos P., Colorado State University (P026, P048, P145, P173, T54, T59)

Kramer, Marianne F., University of South Florida (S09)

Kretsinger, Katrina, CDC (S19)

Kwon, Nam Hoon, Seoul National University (P068, P151)

Kwon, Sung Hee, Hanyang University (P200)

Labuza, Ted, University of Minnesota (S18)

Lamar, Kimberly D., The University of Tennessee-Knoxville (P141)

Lang, Wendy, Alabama A&M University (P167)

Lang, Megan M., University of Georgia (P022)

Lappi, Victoria R., Cornell University (T52)

Larson, Ann E., University of Wisconsin-Madison (P101)

Ledenbach, Lori, Kraft Foods (T09, S11)

Lee, Kyung-Mi, Yonsei University (P202)

Lee, Yih J., Access Business Group (P058)

Lee, Je-Myung, Gyeongsang National University (P199)

Lee, Robin, CDC-NCEH (T39)

Lee, Young duck, Kyungwon University (P123)

Lee, Bong-choon, North Carolina State University (P127)

Lepper, Wendy A., Silliker, Inc. (T05)

Lewandowski, Vickie, Kraft Foods (S06)

Li, Jingkun, Strategic Diagnostics Inc. (P085, P106)

Li, Yanbin, University of Arkansas (P084)

Li, Xin, North Carolina State University (P157)

Lihono, Makuba, University of Arkansas-Pine Bluff (P050)

Lin, Chia-Min, University of Georgia, CFS (P223)

Line, Eric, USDA-ARS-RRC (S15)

Ling, Valerie W., The University of Tennessee-Knoxville (P011)

Lionberg, William C., R & F Laboratories (P154)

Little, Christine L., Communicable Disease Surveillance Centre (P213)

Liu, Xaunli, University of Illinois at Urbana-Champaign (T64)

Lloyd, David C., University of Wales Institute-Cardiff (T19, T44)

Loneragan, Guy H., West Texas A & M University (P063)

López-Malo, Aurelio, Universidad de las Américas-Puebla (P001, P012, P180)

Lowman, Ruff, Canadian Food Inspection Agency (S15)

Mabilat, Claude, bioMérieux (S09)

Mahakarnchanakul, Warapa, Kasetsart University (P288)

Maiden, Martin C. J., University of Oxford (S22)

Majkowski, Jesse, USDA-FSIS (S13)

Maks, Nicole D., NCFST (P207)

Mann, Jason E., Texas Tech University (P148, P158, P159)

Marek, Patrick J., University of Connecticut (T10)

Marshall, Doug, Mississippi State University (S08)

Martinez, Amaury, Universidad Central de Venezuela (P230)

Mathiasen, Lisa A., University of Guelph (T24)

Matthews, Karl R., Rutgers University (P261)

McCullough, Ernie, Triarc Restaurant Group (S04)

McGarry, Sherri, FDA-OFP (S19)

McIntyre, Deborah A., rtech laboratories (P096)

McKnight, Susan K., Quality Flow Inc. (S25)

Medeiros, Lydia C., Ohio State University (S01)

Meldrum, Richard, Public Health Laboratory Service-Wales (P121)

Mena, Kristina D., University of Texas Health Science Center at Houston (S25)

Mendonca, Aubrey F., Iowa State University (S16)

Meyer, Joseph D., Kraft Foods (S23)

Michaels, Barry, Georgia-Pacific Corporation (S03, S05)

Miller, Art, FDA-CFSAN (S13)

Mohamed, Hussein M., Ohio State University (P171)

Mohan Nair, Manoj Kumar, University of Connecticut (P136)

Molins, Ricardo A., Institute of Medicine of The National Academies (T21)

Montville, Rebecca, Rutgers University (P177)

Moon, Hye-kyung, Changwon National University (P203)

Moore, Ginny, University of Wales Institute-Cardiff (P195, T14)

Moorman, Mark, W. K. Kellogg (S10)

Moorman, Mark A., Michigan State University (P217)

Mor, Amram 0., Technion-Israel Institute of Technology (P018)

Morales, Roberta A., Research Triangle Institute (P161, P169)

Murano, Elsa A., USDA (Plenary Session)

Musgrove, Michael T., USDA-ARS (P132)

Nam, Yong Suk, KoGene BioTech., Inc. (P108)

Nanke, Kevin E., SureBeam Corporation (S02)

Nesbitt, Crystal A., FMC Corporation (P216)

Ngutter, Crystal M., University of Vermont (T11)

Niemira, Brendan A., USDA-ARS-ERRC (P253)

Notermans, S., TNO Nutrition and Food Research Institute (S14)

Novak, John S., USDA-ARS-ERRC (S16)

Nychas, George E., Agricultural University of Athens (T36)

Odumeru, Joseph A., University of Guelph (P075)

Oliveira, Manuela, CIISA/Laboratório de Inspecção Sanitária (P282)

O'Neil, Carolyn, Carolyn O'Neil Productions (S24)

Oscar, Thomas P., USDA-ARS (P172, P174, P176)

Otto, Charles S., CDC (S20)

Otwell, Steve, University of Florida (S04)

Oyarzabal, Omar A., Neogen Corporation (T06)

Ozdemir, Feramuz, University of Akdeniz (P194)

Ozogul, Fatih, University of Cukurova (T67)

Pagotto, Franco, Health Canada, Food Directorate (P046, P079, S06)

Palumbo, Mary, California Dept. of Health Services (S19)

Paik, Sujin S., Iowa State University (P185)

Palekar, Mangesh P., Texas A&M University (P238)

Palou, Enrique, Universidad de las Américas-Puebla (P013, P186)

Pangloli, Philipus, University of Tennessee (P061, P092)

Panicker, Gitika, University of Alabama-Birmingham (T04)

Parish, Mickey E., University of Florida (P242)

Park, Kihwan, Chung-Ang University (P249)

Park, J. Douglas, FDA (S19)

Patazca, Eduardo, NCFST, Illinois Institute of Technology (P184)

Penteado, Ana L., Universidade Estadual de Campinas/UNICAMP (P239)

Periago, María Jesús, Murcia University (P284)

Peters, Adrian, University of Wales Institute (S25)

Petersen, Barbara J., Novigen Sciences, Inc. (S23)

Pierre, Pascale M., Michigan State University (P115)

Pillai, Suresh D., Texas A&M University (T27)

Piotrowski, Christine L., Virginia Tech (P247)

Pometto, Tony, NASA Food Technology, Commercial Space Center (S20)

Powell, Mark R., USDA (T62)

Quilliam, Daniela N., CDC-NCEH (T38, T40)

Quintero-Ramos, Armando, Cornell University (P245)

Ransom, Justin R., Colorado State University (T47, T48)

Rasanayagam, Vasuhi, NCFST, Illinois Institute of Technology (P099)

Rash, Vivian Ann, University of Tennessee (P240)

Ravishankar, Sadhana, NCFST (S23)

Redmond, Elizabeth C., University of Wales Institute-Cardiff (P220, P222)

Reimers, Fred, H. E. Butt Grocery Company (S04)

Reynolds, Kelly A., University of Arizona (S25)

Richards, Gary P., Delaware State University (S16)

Richards, Glenner M., University of Georgia, CFS (P236)

Poster Abstracts

Journal of Food Protection, Vol. 66, Sup. A – pp. 59-191

DSC – Developing Scientist Competition

P001 *Escherichia coli* and *Staphylococcus aureus* Inhibition with Ternary Mixtures of Thymol, Carvacrol and Potassium Sorbate

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Phenolic compounds, naturally present in plants, can be used as antimicrobial agents. However, to establish their usefulness, they must be evaluated in combination with other antimicrobials to determine whether there are synergistic effects. Individual and combined effects of thymol (Th), carvacrol (Cr), and potassium sorbate (KS) concentrations on the growth of *Escherichia coli* and *Staphylococcus aureus* were evaluated. Tripticase soy agar was prepared with NaCl and hydrochloric acid to reach a_w 0.99 and pH 4.5, and the necessary amount of Th and/or Cr (0, 15, 30, 45, up to 210 ppm) was added. Over solidified plates of each combination, 50 µL of KS solution (5%) were exponentially deposited using a spiral plater, dried for 2 h, inoculated (10^6 cell/ml) using a swab and making 6 cm long radial marks of every bacteria, incubated at 35°C, and observed after 2 to 5 days. KS inhibitory concentrations were determined by calculating concentration at growth end points. Minimal inhibitory concentrations (MIC) for Th, Cr, and KS were determined, as well as inhibitory concentrations for every ternary antimicrobial mixture. Fractional inhibitory concentrations (FIC) and FIC Index were calculated. MICs were 250 and 600 (KS), 150 and 150 (Cr), as well as 200 and 150 ppm (Th) for *E. coli* and *S. aureus*, respectively. Calculated FIC Index were lower than 0.7. FIC indexes as well as FIC isobolograms (planes deviated to the left of the additive curve) show synergistic effects. Ternary mixtures of phenolic compounds (Th and Cr) with KS drastically reduced the individual inhibitory concentrations, expanding the possibilities to design antimicrobial agents that include naturally occurring ones.

P002 Origanox as a Natural Ingredient to Inhibit the Growth of Foodborne Pathogens

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The Pathogen Reduction Program of the US Department of Agriculture Food Safety and Inspection Service recommends that natural antimicrobial treatments such as herb extracts be included to reduce or inactivate foodborne pathogens. Origanox (OX) is a common herb extract that has been used as a functional ingredient in foods but has not been extensively examined as a possible antimicrobial agent. The objective of this study was to evaluate the effect of OX on the survival and growth of *Escherichia coli* O157:H7, *Salmonella choleraesuis* subsp. *choleraesuis* and *Salmonella* Agona. *E. coli* O157:H7 and *Salmonella*, grown separately in BHI at 37°C for 24 h, were inoculated (final inoculum level of 2 log/ml) into BHI broth OX with different concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0%. Samples were then incubated at 37°C for 8 h. Samples were withdrawn every 2 h during the incubation period and surface plated on EMB agar and XLD agar for enumeration of *E. coli* and *Salmonella*, respectively. Results showed that the addition of 0.2% OX significantly inhibited the growth of pathogenic bacteria when compared to control samples. During the 8 h storage period, populations of bacteria increased by 7.0 log CFU/ml in control samples while bacterial populations in samples with the addition of 0.5% OX treated samples only increased by 2.2 log CFU/ml. When OX at 0.1% was added to BHI agar, at least 2-log reduction in the microbial population was observed. These results indicated the potential applicability of OX as antimicrobial agent for increasing the biosafety of many consumable food products.

P003 Use of Lactoferrin to Inhibit the Growth of Foodborne Pathogens and Meat Spoilage Bacteria

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Lactoferrin (LF), the main iron-glycoprotein present in the milk of mammals, has antimicrobial (AM) effects against a wide range of bacteria, fungi, and parasites. LF antibacterial action is reduced by divalent cations and in some instance by lower temperatures. The objective of this study was to compare the AM activity of LF against *Carnobacterium viridans*, *E. coli* O157:H7 and *Listeria monocytogenes* at their optimal temperatures and 10°C in the presence of 2.5% NaCl in Lauria Broth as a model for cured meat. Sodium bicarbonate (SB) or EDTA were used as ion chelators. By itself, LF at 8 mg/ml killed 4 $\log_{10}$ CFU/ml *C. viridans* within 2 h or 2 days at 30°C or 10°C, respectively. With *E. coli* O157:H7, the reference combination of 32 mg/ml LF plus 0.16 M SB was bactericidal (2 $\log_{10}$ reduction) at 37°C. At 10°C, 32 mg/ml LF alone was bacteriostatic but was bactericidal at 16 mg/ml of LF with 0.04 M SB. Against *Listeria monocytogenes*, 32 mg/ml LF plus 0.16M of SB were bacteriostatic at both 37°C and 10°C. However, after 5 days at 10°C, growth began, reaching 1 $\log_{10}$ CFU by 10 days. Stabilization of LF structure by chelation (EDTA was generally more effective than SB) or other physical means will be key to successful application of LF in food systems as an AM.

P004 Antimicrobial Activity of Cetylpyridinium Chloride against *Listeria monocytogenes* in Ready-to-Eat Meat

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Listeria monocytogenes has emerged as an important foodborne pathogen in the past decade. The effects of cetylpyridinium chloride (CPC) on the inhibition and reduction of *L. monocytogenes* cells were studied in addition to effects on aerobic bacterial populations (APC), lactic acid bacteria (LAB), yeasts and molds, total coliforms, and *E. coli*. Frankfurters were inoculated with *L. monocytogenes* and sprayed with 1% CPC or CPC followed by a water spray treatment. Treatments were applied to the frankfurters by use of a spray cabinet with variable parameters (25, 40, and 55°C spray temperatures; 20, 25, and 35 psi spray pressures; and 30, 40, and 60 s time of exposure). No differences ($P > 0.05$) were observed between the different spray parameters. A 2.5 $\log_{10}$ CFU/g reduction of *L. monocytogenes* was achieved by 1% CPC. Bacteriostatic effects of 1% CPC were observed as a result of inhibition ($P = 0.05$) of *L. monocytogenes* on surfaces of frankfurters, polish sausages, and roast beef stored for up to 42 days at 0°C and 4°C. Similar effects were observed for APC, LAB, yeasts and molds, total coliforms, and *E. coli*. Spray treatment (1% CPC) did not ($P > 0.05$) affect the color of ready-to-eat meat products as a result of up to 42 days of storage at 0°C and 4°C. Hardness of 1% CPC treated frankfurters was significantly ($P = 0.05$) lower than non-treated frankfurters, but no effect ($P > 0.05$) of treatment was observed on the hardness of polish sausages and roast beef stored for 42 days.

P005 Antimicrobial Effects of Colloidal Silver on Beef Inoculated with *Salmonella* spp.

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Colloidal silver is commonly used as a natural antimicrobial agent containing silver molecules between 1 to 100 nm in size in deionized water. Colloidal silver is produced from silver electrodes, which creates silver molecules primarily in a cationic form. This study focused on the efficacy of a colloidal silver solution utilized as an antimicrobial rinse against *Salmonella* spp. on beef. Samples were fabricated into 180 cm^2 pieces and inoculated with a five strain cocktail of *Salmonella* spp. at 10^6 CFU/cm^2 and 10^4 log CFU/cm^2 solutions respectively. Treatments of either 32 ppm of colloidal silver or deionized water were applied and compared to untreated samples. Treatments were applied to beef at 20 psi from a distance of 13 cm using a spray wash cabinet for 15 s. Duplicate 2.5 cm^2 core samples were randomly drawn at 0 min, 20 min, 1 h and 4 h. Surviving bacterial species were enumerated in duplicate on differential and recovery media. Samples inoculated at 10^4 CFU/cm^2 of *Salmonella* spp. and treated with colloidal silver demonstrated a 2.0 log CFU/cm^2 reduction after 4 hrs when compared to untreated samples ($P > 0.05$). Furthermore samples inoculated at 10^6 CFU/cm^2 of *Salmonella* spp. and treated with colloidal silver showed a 1.5 log CFU/cm^2 reduction after 4 h when compared to untreated samples ($P > 0.05$). Additionally, no significant differences were noted between samples treated with deionized water and those samples receiving no treatments.

P006 Influence of EDTA on the Antimicrobial Efficacy of Thai Spices

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There is a need to evaluate and improve the antimicrobial efficacy of spices and their essential oils in foods. EDTA, a chelator, is known to potenti-

ate the activity of food antimicrobials. Therefore, the objective of this study was to investigate the effect of EDTA on the antimicrobial efficacy of Thai spices including ginger (*Zingiber officinale Rosè*), fingerroot (*Boesenbergia pandurata Holtt*), and turmeric (*Curcuma longa Linn*) against *Listeria monocytogenes* and *Salmonella* Typhimurium DT 104. Spices were obtained fresh from a market in Bangkok or as essential oils (TCFF, Ayutthaya, Thailand). Fresh spices were extracted with 50% ethanol. A microwell assay was used to study the activity of extracts with EDTA against strains of both bacteria. The effect of essential oils with EDTA was determined using an agar dilution assay. A model food system (whole milk with essential oils and EDTA) was inoculated with *S.* Typhimurium and growth monitored for 7 days at 10°C. *L. monocytogenes* were more sensitive to 10% extracts of ginger and fingerroot than *S.* Typhimurium. Turmeric extract had little antimicrobial effect. EDTA did not enhance the antimicrobial activity of extracts against *L. monocytogenes.* In contrast, EDTA had positive effects with the extracts and essential oil of fingerroot (EOF) against *S.* Typhimurium. The antimicrobial efficacy of 0.3% EOF with 500 µg/ml EDTA against *S.* Typhimurium was reduced but not eliminated in whole milk. Therefore, EDTA may enhance antimicrobial activity of native spice extracts and essential oils and could assist in control of foodborne pathogens.

P007 Evaluation of Antimicrobial Packaging Materials and Modified Atmosphere Packaging for the Preservation of Foods

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Allyl ithocyanate in horseradishes has an inhibitory effect against microorganisms. Packaging materials containing Western wasabi extract have been commercialized in Japan to extend the shelf life of products. The application of such packaging materials is not very common in the United States; however, there is increasing interest in recent years. In this study, the efficacy of the Western wasabi extract incorporated into packaging film or pad was evaluated against *Escherichia coli* on plates, *Listeria monocytogenes* on plates and smoked salmon, and *Salmonella* Enteritidis on chicken. In addition, the synergistic effect of modified atmosphere packaging (MAP) and antimicrobial packaging materials was evaluated. The antimicrobial film under air packaging suppressed 90% of *E. coli* on plates at 20 and 35°C. The MAP significantly enhanced the inhibitory effect of the film at 20°C, but not at 35°C. Unlike for *E. coli*, the inhibitory effect of the film against *L. monocytogenes* at 20°C was greater in the absence of the gas. On smoked salmon, gas mixture alone was sufficient to suppress the growth of *L. monocytogenes* at 7 and 15°C, and the addition of antimicrobial pads did not provide further benefits. On chickens, the antimicrobial pad failed to inhibit the growth of *S.* Enteritidis at 15 and 20°C. At 7°C, *S.* Enteritidis counts increased after 8 days but returned to the initial levels after 14 days. The antimicrobial packaging materials showed some potential for food preservation; however, their design may need some improvement to obtain greater inhibitory effects.

P008 DSC Antibacterial Effect of Black Seed Oil on *Listeria monocytogenes*

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Listeria monocytogenes is a major foodborne pathogen in the United States. Effective methods for reducing *L. monocytogenes* in foods would reduce the likelihood of foodborne outbreaks of listeriosis, and decrease economic losses to the food industry. *Nigella sativa* is a herbaceous plant whose seeds (black seed) have been used as a spice and condiment in foods in the Middle East. The objective of this study was to determine the antibacterial effect of black seed oil (BSO) on twenty strains of *L. monocytogenes* by disc diffusion method. A population of 7.0 log CFU of each strain of *L. monocytogenes* was inoculated on duplicate plates containing antibiotic medium 1 agar. The plates were allowed to dry at room temperature for 10 min. Three discs (6 mm diameter), each impregnated with 10 ml of black seed oil, vegetable oil (oil control), or gentamicin (positive control), were placed on each inoculated plate. The plates were incubated at 37°C for 24 h, and were observed for zones of *L. monocytogenes* growth inhibition. The study was replicated thrice. BSO exhibited a strong antibacterial activity against all the strains of *L. monocytogenes*, yielding a significantly ($P < 0.01$) larger inhibition zone than that of gentamicin. The mean zones of inhibition produced by BSO and gentamicin were 31.50 ± 1.0 and 14.80 ± 0.50, respectively. The vegetable oil had no inhibitory effect on *L. monocytogenes.* Results indicate that BSO could potentially be used to inhibit *L. monocytogenes*, but appropriate applications in foods need to be validated.

P009 DSC Protamine's Antimicrobial Activity against *Escherichia coli* Depends upon Cell Envelope Structure and Electrostatic Interactions

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Protamine, a cationic antimicrobial peptide derived from herring sperm, may be a useful food additive. To optimize protamine for food applica-

tions, more knowledge is required about its mechanisms of action. Since *Escherichia coli* shows a wide range of susceptibility to protamine, this study examines how the structure of the *E. coli* cell envelope affects protamine sensitivity. Minimum inhibitory concentrations (MICs) for protamine were determined for 28 *E. coli* by microtitre broth dilution assay, using the redox dye Alamar Blue. Strains represented different O-antigen types (including rough and smooth), different types of core-lipopolysaccharide (LPS) and R1-core-LPS mutants. MICs ranged from 78-2500 ìg/ml. Smooth *E. coli* were up to 4 times more resistant than rough strains, indicating that the O-antigen provides a rate-limiting barrier to protamine. R1-core-LPS mutants missing at least one phosphate group in their core were twice as resistant as those retaining both phosphate groups, suggesting that protamine activity depends on electrostatic interactions with LPS. To investigate how *E. coli* O-antigen affects survival, a smooth and a rough *E. coli* strain were treated with protamine at MIC. Survival was monitored over 24 h by plate counting and by fluorescence microscopy, using the BacLight Live/Dead stain. Plate counting showed significant inhibition for both strains. However, the percentage of living cells determined by microscopy was identical to the untreated control for the rough strain, while it was clearly reduced for the smooth strain. Thus, factors other than O-antigen and charge in the core-LPS must influence the effect of protamine on *E. coli.*

P010 Withdrawn

P011 DSC Antimicrobial Activity of Selected Chemical Components from Essential Oils against *Salmonella* Typhimurium and *Listeria monocytogenes*

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The antimicrobial activity of essential oils may be due to two or more chemical components in the oil. The objective of this study was to evaluate the antimicrobial activity of selected essential oil components against *Salmonella* Typhimurium and *Listeria monocytogenes* using microbroth and macrobroth dilution assays. Carvacrol, beta-caryophyllene, trans-cinnamaldehyde, eugenol, (S)-(-)-limonene, linalool, (1R)-(+)-alpha-pinene, (1S)-(-)-alpha-pinene, rhodinol, and thymol were tested using a 24-h inoculum diluted to log 5.7 CFU/ml in phosphate-buffered brain-heart infusion broth (pH 7.2) with 1% Tween 20 in microtiter plates (microbroth) and glass tubes (macrobroth). The most effective essential oil components against *L. monocytogenes* using the microbroth dilution method were carvacrol (minimum lethal concentration=3000 ppm), cinnamaldehyde (MLC=1200 ppm), eugenol (MLC=2600), and rhodinol (MLC range= 2000-2200). In contrast, lower concentrations inactivated *S.* Typhimurium since concentrations of 1400 ppm carvacrol, 400 ppm cinnamaldehyde, 1400 ppm eugenol, and 1400-2000 ppm linalool and rhodinol were bacteriocidal against *S.* Typhimurium using the microbroth dilution assay. Beta-caryophyllene, limonene, (1R)-(+)-alpha-pinene, (1S)-(-)-alpha-pinene, and thymol were not inhibitory to *L. monocytogenes* and *S.* Typhimurium at 2000 ppm. Components from essential oils, such as rhodinol and linalool, are hydrophobic and some may react chemically with the polystyrene microtiter plates, thus causing variable minimum lethal concentrations. The use of glass tubes for the macrobroth dilution assay produced more consistent results and is recommended. The MLCs of chemicals from essential oils against *S.* Typhimurium were 400 ppm of cinnamaldehyde and 1400 ppm of carvacrol, eugenol, linalool and rhodinol as determined by the macrobroth dilution assay. Since selected components of essential oils were inhibitory to *S.* Typhimurium and *L. monocytogenes,* combinations of these components may be useful as antimicrobials and should be evaluated.

P012 Antimicrobial Activity of Potassium Sorbate and Phenolic Compound Mixtures

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A wide range of phenolic compounds naturally occurring in plants are being evaluated as antimicrobials. Among them, thymol (Th), eugenol (Eu), and carvacrol (Ca) have demonstrated antimicrobial activity. However, data on the effect of combinations with traditional antimicrobial agents such as potassium sorbate (KS) is scarce. The effect of selected combinations of Th, Eu or Ca (50,100, 150, up to 350 ppm) with KS (50, 100, 150 up to 800 ppm) on *Staphylococcus aureus, Escherichia coli, Listeria innocua* and *Salmonella* Typhimurium growth, inoculated in a_w 0.99 or 0.95 - pH 3.5 or 4.5 tripticase soy agar (TSA) were evaluated. For all bacteria, TSA prepared with each a_w, pH and antimicrobial mixture was spiral plated with 10^6 cells/mL suspension, incubated at 35°C, observed and counted after 3 to 5 days. Minimal inhibitory concentration (MIC) was defined as the minimal required individually to inhibit growth (counts < 10 CFU/mL). Fractional inhibitory concentrations (FIC) were calculated from individual and inhibitory combinations; also FIC index of each antimicrobial mixture was computed. For every antimicrobial, MICs were higher for *S. aureus* inhibition than for the studied bacteria. Several mixtures of KS and Th or Eu or Ca inhibit the four bacteria with lower

concentrations than those needed when utilized alone. FICs and FIC index show synergism depending on the antimicrobial concentration in the mixture, a_w and pH. Synergistic combinations with FIC index < 0.6 included 100 ppm Th, Eu or Ca with 100 or 200 ppm KS, depending on a_w and pH.

P013 Carvacrol, Citral, Eugenol, Potassium Sorbate, Sodium Benzoate, Thymol, and Vanillin Inhibitory Concentrations of *Zygosaccharomyces bailii* Growth Determined by Probabilistic Modeling

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Probabilistic microbial modeling using logistic regression was used to predict carvacrol, citral, eugenol, potassium sorbate, sodium benzoate, thymol, and vanillin inhibitory concentrations on *Zygosaccharomyces bailii* growth in the presence of growth controlling factors such as a_w (0.99, 0.97 or 0.95) and pH (4.5 or 3.5). For every antimicrobial, a_w and pH adjusted Sabouraud broth was prepared and selected antimicrobial concentrations (ranging from 0 to 2600 ppm, with increments of 25, 50 or 100 ppm depending on the antimicrobial agent) added. Micro-titer wells (5 replicates) were inoculated (2 µL, 10^6 cell/mL suspension), incubated and observed up to 10 days. If growth was observed the response was 1, if not it was 0. For every antimicrobial, backward stepwise logistic regression was used to develop a simplified model able to predict probability of yeast growth. Each model includes the significant ($P < 0.05$) effect of each independent variable as well as a_w-pH and pH-antimicrobial concentration interactions. From a total of 4230 observations, in > 50% of the cases growth was observed. The models can be used to calculate critical values of every antimicrobial concentration needed to inhibit yeast growth for different probabilities. The reduction of pH increased the number of combinations of a_w and antimicrobial concentration with probabilities to inhibit growth higher than 0.95. With a probability of growth of 0.05, antimicrobial inhibitory concentrations were higher as a_w and pH increased. Antimicrobial agent concentration critical values, calculated for selected probabilities of growth, can be used to predict inhibitory concentrations for different a_w and pH values.

P014 Shiga Toxin-producing *Escherichia coli* in Nevada Sheep

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Worldwide, shiga toxin-producing *Escherichia coli* (STEC) have been recognized as the cause of many sporadic cases or major outbreaks of human illnesses involving consumption of contaminated meat, especially beef. Although sheep products have not been linked to reported human illnesses, their role as a food safety risk factor should not be ignored. The objective of this study was to assess STEC prevalence in two groups of ewes (20 each) grazing an irrigated pasture or arid range in a western US environment (Nevada) over one year (summer, 1999 to summer, 2000). A random sample (n = 504) of potential STEC isolates was tested for verotoxicity and was screened for presence (polymerase chain reaction) and expression (VTEC-reversed passive latex agglutination assay) of the toxin genes (i.e., ST1 and ST2). Forty-one STEC isolates (16 having only the ST1 gene and 25 having both ST1 and ST2 genes) were detected in both groups of ewes. Except for seven isolates, the genotype and phenotype data matched. All isolates (non-motile [H-]) were non-O157:H7 STEC (i.e., O91:H- [n = 25], O128:H- [n = 9], and untypeable ones [n =7]). More infected ewes (9 vs 3) and different STEC strains were found in the irrigated pasture than in the arid range. Because our ewes were shedding two STEC serotypes known to cause human illnesses, it is beneficial to identify STEC-positive sheep before slaughter as an initial control point before the animals enter the food chain.

P015 DSC A PCR-based Method for the Rapid Detection of the Genus *Listeria* and the Species *Listeria monocytogenes* in Food Products

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The genus *Listeria* comprises six closely related species of which only *Listeria monocytogenes* is a human pathogen. Rapid and sensitive detection of *L. monocytogenes* is important in the food industry as well as in medical diagnosis. We have developed a PCR-based method for the rapid, specific, and sensitive detection of *L. monocytogenes* in food products. The PCR reaction is based on DNA sequences and primer pairs that are found within the 16S subunit of the rRNA genes and are specific to the *Listeria* genus and to *L. monocytogenes* within the *Listeria* genus. The primers for the *Listeria* genus and *L. monocytogenes* species were used in the same reaction mix for their simultaneous detection. In addition, a pair of bacterial universal primers that amplify any bacterial source were developed as a positive control. For detection from food products, the method includes selective enrichment for Listeria, followed by DNA extraction, and a specific PCR reaction. The method detects 1 to 5 CFU in a 25 g sample in 24 h or less. It can be easily incorporated into routine screening of diverse food products, and readily adapted for clinical usage.

P016 DSC Biofilm Forming Potential of *Listeria monocytogenes* Isolates on Stainless Steel Using Two Different Media

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Isolates of *Listeria monocytogenes* may exhibit different abilities to produce biofilms in a food processing plant. In order to test this hypothesis, biofilms of 30 isolates of *L. monocytogenes* were grown on stainless steel 304 type 4b in Tryptic Soy Broth [TSB] or Diluted (1:10) Tryptic Soy Broth [DTSB]. The biofilms were stained with bisBenzimide H 33258 (Hoechst 33258), and ten epi-fluorescent images of each biofilm were collected. The percent of the area covered by cells was calculated. Data from 3 reps were analyzed with One-way ANOVA and Duncan's Multiple Range test using SAS. For biofilms grown in TSB, 28 of the isolates produced biofilms (> 4% area covered) versus 15 for biofilms grown in DTSB. However of those 15 isolates grown in DTSB, 11 isolates produced more biofilm than when grown in TSB. Conversely, only 8 isolates grown in TSB produced more biofilm than when grown in DTSB. Of the biofilms grown in TSB, 4 were significantly greater than the rest, and 3 of these were serotype 4b. Conversely, the top 7 biofilms grown in DTSB were serotype 1/2a. Twelve of the 15 isolates that produced biofilms (> 4% area covered) were serotype 1/2a, and 13 of the 15 fifteen non-biofilm producing isolates were serotype 4b. These data suggest that some *L. monocytogenes* isolates are adapted for biofilm growth in certain niches, and when transferred may not immediately adapt to a new environment. These data also suggest that serotype is associated with this adaptation.

P017 Effect of Natural Antimicrobials on *Escherichia coli* O157:H7 in Refrigerated MAP Ground Beef

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Two naturally occurring antimicrobial agents were tested in packages of refrigerated ground beef for their ability to reduce the viability of *E. coli* O157:H7 during storage. The natural antimicrobials allyl isothiocyanate and *Lactobacillus reuteri* were tested separately and together for their action against a cocktail of 5 strains of *E. coli* O157:H7 in ground beef held at 4°C for 25 days. Ground beef was inoculated with low (3 log CFU/g) or high (6 log CFU/g) levels of the *E. coli* O157:H7 mixture. The beef was treated with AITC (about 1300 ppm) and/or *Lactobacillus reuteri* along with 250 mM glycerol/kg meat at two levels (3 log CFU/g and 6 log CFU/g) and according to a design that yielded 8 control plus 10 different treatments. Samples were analysed for *E. coli* O157:H7 survivors, number of total bacteria and lactic acid bacteria on days 0 to 25 at 5 day intervals. *Lactobacillus reuteri* with glycerol at both input levels killed *E. coli* O157:H7 at both inoculated levels before day 20. AITC completely eliminated *E. coli* O157:H7 when present at the low inoculum level (3 log CFU/g) and reduced viability >4.5 log CFU/g at the high inoculum level (6 log CFU/g) by the end of the storage period. Combination of *Lactobacillus reuteri* with AITC did not yield an additive effect against *E. coli* O157:H7 viability. *Lactobacillus reuteri* in the presence of glycerol was highly effective against *E. coli* O157:H7 in ground beef during refrigerated storage (4°C) in modified atmosphere packages.

P018 Activity of Dermaseptin Derivatives against Foodborne Pathogens

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Peptide-based antimicrobials are emerging as a highly promising class of broad-spectrum antimicrobial agents due to their potent and rapid cytolytic activity to which pathogens are presumably unable to develop resistance. We recently produced a peptide library based on dermaseptin S4 derivatives and showed the library to contain potent antimicrobial peptides, with demonstrable selective activity both in culture media and in animals. Here, we examined the peptides potential efficacy in food preservation. The library was screened for growth inhibitory activity in culture, against a variety of bacterial species that are relevant to food pathogenicity, including the Gram-negative foodborne pathogens *Escherichia coli* O157:H7 and *Salmonella enterica* (serotypes Typhimurium, Stanley, and Virchow) as well as the Gram-positive pathogen *Listeria monocytogenes*. The data enabled the identification of various active peptides with MIC in the range of 2 to 8 μg/ml. In commercial apple juice (pH = 3), short derivative displayed improved potency compared with the parent peptide with rapid bactericidal activity against *E. coli* O157:H7, reducing its CFUs by more than seven log units after 15 min incubation. Based on the screen results, active peptides were selected for further in-depth characterization including investigation of the peptide's effect with respect to stereospecificity, pH, salt and temperature dependences both in culture conditions and in commercial apple juice. Overall, the data was consistent with a membrane-active receptor-independent mechanism of action and indicated the potential usefulness of such peptide-based antimicrobials as food preservatives.

P019 An Exopolysaccharide, Colanic Acid, Production by Shiga-toxin-producing and Enterohemorrhagic *Escherichia coli*

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Shiga-toxin-producing and enterohemorrhagic *Escherichia coli* (STEC and EHEC) secrete a variety of exopolysaccharides (EPS). The principal component of the slime EPS is colanic acid (CA), which contains fucose, glucuronic acid, glucose, and galactose with acetyl and pyruvyl groups. Although many strains of STEC and EHEC are capable of CA production, it is not known whether such production is influenced by their growth conditions. The objectives of this study were to quantify the CA produced by strains of STEC and EHEC, and to determine the effects of medium pH and incubation temperatures on CA production. Strains of STEC and EHEC were retrieved from frozen stock, and propagated on Bacto M9 casamino acid agar. A loopful of each culture was transferred to minimal glucose agar (MGA) with pH 7.5, 6.5, 5.5 or 4.5. The inoculated plates were incubated at 37, 22, 15 or 10°C for 2, 3, 4, and 10 days, respectively. The cultures were harvested with 10 ml of 0.15 M NaCl. The amounts of uronic acid in the cell suspensions were determined by a colorimetric assay with glucuronic acid as a standard. The quantities of the proteins in the suspensions were measured using a BCA assay to account for differences in quantities of bacterial growth. The uronic acid and protein ratios (UA/P) were used to express the levels of CA produced by strains of STEC and EHEC on pH-adjusted MGA at various incubation temperatures. The research revealed that the UA/P ratios of 59 STEC and EHEC ranged from 33 to 377.3. The UA/P ratios of the same *E. coli* O157:H7 strain varied substantially under different growth conditions. The greatest amount of CA was produced on MGA with pH 7.5 at 10°C, followed by 15°, 22°, and then 37°C, with few exceptions. These results suggest that the amounts of CA produced by STEC and EHEC vary to a great extent, and CA production in *E. coli* O157:H7 is influenced by medium pH and incubation temperatures.

P020 DSC Adaptation to Low pH Changes; Membrane Lipid Composition, Verotoxin Secretion, and Acid Resistance of *Escherichia coli* O157:H7

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The objective of this study was to measure changes in *E. coli* O157:H7 membrane lipid composition, verotoxin secretion, and acid resistance in simulated gastric fluid (pH 1.5) of various pH adapted cells. *E. coli* O157:H7 (HEC), *E. coli* O157:H7 *rpoS* mutant (HEC-RM), and nonpathogenic *E. coli* (NPEC) were acid adapted by incubation (37°C) in TSB at pH 7.3 for 18 h, then transferred to pH 6.0 or 8.3 for 18 h, followed by an additional 24 h at pH 5.0 or 9.0. Medium pH was adjusted with HCl or NaOH. After incubation at each pH, populations were measured for acid resistance (D-value) in simulated gastric fluid, membrane lipid composition, and intracellular and extracellular verotoxin concentrations. Membrane lipids were measured by gas chromatography and verotoxin was measured by optical density (450 nm) of ELISA reactions. The ratio of palmitic acid (16:0) to *cis*-vaccenic acid (18:1ω7c) increased at acidic pH, causing a decrease in membrane fluidity. HEC adapted at pH 8.3 and HEC-RM adapted at pH 7.3 had highest verotoxin concentrations (O.D. 5.01 and 2.96, respectively). In addition, the ratio of extracellular to intracellular verotoxin concentration decreased at acidic pH. Regardless of strains, D-values of acid adapted cells increased and HEC adapted at pH 5.0 had highest D-value of 21.8 min. The decrease in membrane fluidity significantly increased intracellular verotoxin concentration and acid resistance, but decreased extracellular verotoxin concentration. The deletion of the general stress response gene (*rpoS*) did not affect the changes in membrane fluidity, verotoxin concentration, or acid resistance.

P021 DSC Effect of Ultrasonication and Sodium Chloride Concentration on Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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A study was conducted to determine the effect of high-intensity ultrasound application and NaCl concentration on inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Test strains were cultured, washed, and resuspended (8 to 9 log CFU/mL) in 99 mL of sterile aqueous solutions containing 0, 0.5, 1.0, 2.0, and 5.0% NaCl. Inoculated solutions were sonicated under isothermal conditions (20°C) for 1, 5, and 10 min at power settings of 18, 48, and 111 Watts. After treatment, samples were diluted, plated onto tryptic soy agar, and incubated for 24 h at 35°C (*E. coli* O157:H7) or 48 h at 30°C (*L. monocytogenes*). NaCl concentration or treatment time at 18 and 48 Watts had little or no effect on survival of *E. coli* O157:H7 (< 1 log reduction). At 48 and 111 Watts, NaCl concentrations > 0.5% adversely affected survival (1.1 – 1.6 log reduction). For *L. monocytogenes*, reductions of

< 0.8 log were observed after 10 min of treatment at 111 Watts. NaCl concentration had no effect on survival of *L. monocytogenes*, regardless of sonication time or power. While increased solute concentration typically enhances microbial thermotolerance, this study demonstrates that this may not be the case with ultrasonication. We suggest that the increased deactivation efficiency in the presence of salts may be attributed to enhanced cavitation. These results provide valuable insight into both the fundamental theoretical nature of microbial deactivation by ultrasound and the practical aspect of inactivating microorganisms in products such as low water activity foods.

P022 DSC Comparison of Inoculation Method and Drying Time on Survival and Recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* Inoculated onto Raw Tomatoes and Lettuce

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A study was undertaken to evaluate the effect of method of inoculation and drying time on survival and recovery of foodborne pathogens inoculated onto the surface of tomatoes and iceberg lettuce. Five-strain mixtures of *Escherichia coli* O157:H7, *Salmonella*, or *Listeria monocytogenes* were applied to tomatoes and lettuce by dip, spot, or spray inoculation methods. Inocula were dried for 1 or 24 h at 22°C on tomatoes or 2 h at 22°C, followed by 22 h at 4°C, on lettuce before treating with water (control) or chlorine (200 µg/ml). Significantly ($P = 0.05$) higher populations of *E. coli* O157:H7 and *Salmonella* were recovered from tomatoes and lettuce inoculated by dipping compared to spot or spray inoculation. Populations of the two pathogens recovered from tomatoes and lettuce inoculated by spot and spray methods were not significantly different. Significantly different populations of *L. monocytogenes* were recovered from tomatoes (dip > spot > spray) and lettuce (dip > spray > spot). Populations of pathogens recovered from tomatoes were significantly higher when inocula were dried for 1 h compared to 24 h. Populations recovered from lettuce after drying inocula for 2 h at 22°C were significantly higher or equal to populations recovered after drying for 2 h at 22°C followed by 22 h at 4°C. Significant differences were observed in populations of all pathogens recovered from treated tomatoes and lettuce (water > chlorine). Results indicate that inoculation method, drying time, and treatment affect survival and/or recovery of foodborne pathogens inoculated onto the surface of tomatoes and lettuce.

P023 DSC Changes in the Acid Tolerance of *Escherichia coli* O157:H7 as Affected by Acid Adaptation Procedures

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Acid tolerance response (ATR) may be induced when an organism is exposed to environments promoting activation of low pH survival inducing systems. Several procedures (acid adaptation, acid habituation, and acid shock) have been used to induce acid resistance in *Escherichia coli* O157:H7. This study evaluated ATR in *E. coli* O157:H7 as affected by various acid resistance inducing procedures as well as strain, inoculum level, growth phase, acid type, and glucose concentration. Twelve growth conditions in tryptic soy broth were based on glucose levels (0%/-G, 0.25%/G, 1%/+G) and adjustment of pH (7.0, 5.0, or 5.5) with hydrochloric (HCl) or lactic (LA) acid, to assess ATR at different incubation (37°C) times. Strain ATCC 43895 had a higher ATR than ATCC 43889. Lower ATR was found in cultures originating from a lower compared to a higher initial inoculum. Exponential phase cells expressed the lowest ATR, stationary phase cells the highest ATR, and slight to moderate reductions in the ATR of *E. coli* O157:H7 were seen in the declining phase. Acid tolerance was lower when *E. coli* O157:H7 was challenged with LA (pH 3.5) compared to challenging with HCl (pH 2.0). Glucose (G and +G) induced an ATR during stationary phase. An ATR was induced with or without glucose in broth reduced to pH 5.0 or 5.5. The effect of broth pH on ATR, from lowest to the highest, was LA pH 5.0 > LA pH 5.5 > HCl pH 5.0 > pH 7.0. These results indicate that many factors influence the ATR of *E. coli* O157:H7 and care should be taken to select procedures that simulate the system being studied.

P024 Comparison of Media, Incubation Time, Temperature, and Environmental Conditions on the Recovery of Heat-injured *Escherichia coli* O157:H7 in a Broth System

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Escherichia coli O157:H7 has been implicated in many foodborne outbreaks. Environmental stresses such as heat, cold, and chemicals can injure cells and may cause varying results among media. Recovery media may be better able to resuscitate stressed cells. Research was conducted to determine the optimal medium for recovery of heat-injured *E. coli* O157:H7 with consideration to incubation

time, temperature, and environment. A heating inactivation profile was used to mimic directly acidified meat snack sticks. Samples were taken at 3 different times of the profile to ensure heat injury along with an initial inoculum level. These samples were plated on six different media: MEMB (modified eosin methylene blue), MSA (MacConkey sorbital agar), PRSA (phenol red sorbital agar), TAL [Thin Agar Layer method (MSA overlaid with TSA prior to plating)] TSAOV (Tryptic soy agar overlaid with MSA following plating) and TSAPA (tryptic soy agar with 0.1% pyruvic acid) and was held at different temperatures (20, 25, and 37°C) and environmental conditions for incubation (aerobic and anaerobic chambers). Plates were enumerated 24 and 48 h after incubation. The highest recovery media for heat-injured cells at 24 h was PRSA at 37°C grown anaerobically followed by TSAPA at 37°C in an aerobic environment. After 48 h incubation, TSAPA resuscitated the most injured cells at 25°C in anaerobic conditions followed by PRSA at 37°C in an aerobic environment. Overall verification of media proves heat-injured bacteria need different incubation temperatures and environments for optimal recovery of cells.

P025 Protective Effect of *Escherichia coli* O157:H7 Colanic Acid to Osmotic Shock and Oxidative Stress

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Many strains of *Escherichia coli* O157:H7 produce under stress an exopolysaccharide (EPS) composed of colanic acid (CA), and form mucoid colonies on minimal glucose agar (MGA) at ambient temperature. Previous research conducted in our laboratory involving a CA proficient (W6-13) and a CA-deficient (M4020; *wcaD::Ekan*^{r}) strain of *E. coli* O157:H7 revealed that CA conferred acid and heat tolerance to *E. coli* O157:H7. Cells covered with CA were more persistent during acid (pH 4.5, 5.5, and 6.5) and heat (55° and 60°C) treatment. The goal of this research was to demonstrate the effect of CA on survival of *E. coli* O157:H7 under osmotic shock and oxidative stress. Cells of W6-13 and M4020 were exposed to various concentrations of NaCl (0.5, 1.5, and 2.5 M) and H_2O_2 (0, 10, and 20 mM) in minimal glucose broth (MGB) at 22°C. Viable counts of *E. coli* O157:H7 were determined within 48 h of the osmotic shock and 3 h of the oxidative stress. The results showed that the survival of W6-13 and M4020 was not significantly influenced by 0.5M NaCl ($P > 0.05$). In MGB containing 1.5 M NaCl, the populations of M4020 decreased 3.77 $\log_{10}$ CFU/ml after 48 h at 22°C, whereas the counts of W6-13 declined only 1.72 $\log_{10}$ CFU/ml, significantly different from its CA-deficient mutant ($P < 0.05$). W6-13 and M4020 were both inhibited in MGB containing 2.5 M NaCl. However, there was a 2.69-$\log_{10}$ CFU/ml difference in their populations at the end of the 48 h treatment ($P < 0.05$). During the 3 h treatment with 10 or 20 mM H_2O_2 at 22°C, the populations of W6-13 remained approximately unchanged. However, the survival of M4020 was affected. While the counts of M4020 declined only slightly (0.81 $\log_{10}$ CFU/ml) in MGB containing 10 mM H_2O_2, complete inactivation (9.04 log reduction) was observed under the treatment of 20 mM H_2O_2. The differences in survival between W6-13 and M4020 in MGB containing 10 or 20 mM H_2O_2 were significant, with P values of 0.04 and 0.05, respectively. These results suggest that W6-13 was more tolerant than M4020 to osmotic shock and oxidative stress, and CA plays a vital role in protecting *E. coli* O157:H7 from these stresses.

P026 Survival and Growth of *Escherichia coli* O157:H7 on Fresh Beef Inoculated before and after Decontamination with Hot Water and Lactic Acid in Different Sequences

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Interventions such as hot water and organic acid solutions are used to reduce the prevalence of *Escherichia coli* O157:H7 on beef carcasses. Research is needed, however, to evaluate the effects of such treatments on pathogen survival during product storage. This study evaluated use of hot water (HW) and lactic acid (LA) alone and in combination for their ability to control, during storage, *E. coli* O157:H7, inoculated both pre- or post-decontamination. Pieces (5 × 2.5 × 1 cm) of beef round were inoculated with a five-strain mixture of stationary phase *E. coli* O157:H7 grown in tryptic soy broth without dextrose, and treated by immersion for 30 s in HW (75°C), 2% LA (55°C), and combinations of these (HW/LA and LA/HW). Untreated and treated beef pieces were stored aerobically at 4°C, 10°C or 25°C for 15, 10 and 5 days, respectively. Bacteria were enumerated with sorbitol MacConkey agar supplemented with Cefixime-Tellurite and tryptic soy agar. Samples treated with HW had similar populations as untreated samples during storage at all temperatures. Population trends were similar in samples inoculated pre- and post-treatment with LA. The HW/LA treatment was the most effective in controlling *E. coli* O157:H7 and maintained populations >1.5 log CFU/cm^2 lower than untreated samples. LA/HW treated samples reached similar final populations (8.64 to 9.22 log CFU/cm^2) as the untreated at 25°C; however, stationary phase was delayed by 3 days. Storage at 4°C resulted in population reductions.

Selecting the proper sequence of decontamination with HW and LA may optimize control of *E. coli* O157:H7 during storage of beef inoculated pre- or post-decontamination.

P027 Acid Tolerance of *Escherichia coli* O157:H7 during Aerobic Storage at 4°C, 10°C and 25°C of Beef Treated with Hot Water and Lactic Acid

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Treatment of beef carcasses with hot water and organic acids can effectively reduce the prevalence of *Escherichia coli* O157:H7. However, the use of sub-lethal treatments may select for stress-adapted organisms. This study evaluated the acid tolerance response (ATR) of *E. coli* O157:H7, inoculated on beef treated with hot water (HW) or lactic acid (LA), during aerobic storage at 4°C, 10°C or 25°C. Beef round pieces (5 × 2.5 × 1 cm) were inoculated (pre- or post-treatment) with a five strain composite of stationary phase *E. coli* O157:H7 grown in tryptic soy broth without dextrose (TSB-G) and treated by immersion in HW (75°C) or 2% LA (55°C) for 30 s. The ATR of *E. coli* O157:H7 on beef pieces was assessed on day 0 and on days 7 and 14 (4°C), 5 and 10 (10°C), and 2 and 4 (25°C) using TSB-G of pH 2.43 to 2.51 (HCl) for up to 240 min. Bacteria on LA-treated samples generally expressed a higher ATR than on HW-treated samples. Populations on untreated samples (0.98 to 2.23 log CFU/cm^2 reduction) generally expressed a higher or equivalent ATR compared to LA and HW treatments (0.78 to 3.40 log CFU/cm^2 reduction). The ATR of populations from pre- and post-treated samples was dependent on treatment, temperature, and length of storage. An increase in the ATR of *E. coli* O157:H7 was observed after storage at 25°C and 10°C. Populations of samples stored at 4°C for 14 days expressed an ATR similar to that at day 0; however, trends among treatments were different. The generally higher ATR of untreated samples indicates that decontamination with HW or LA may not increase the acid tolerance of *E. coli* O157:H7.

P028 DSC The Effect of Simulated Spray-chilling on Acid-habituated and Non-acid-habituated *Escherichia coli* O157:H7 Cells Attached to Beef Carcass Tissue

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This study evaluated chemical solutions currently used for carcass decontamination, as well as novel solutions, for their effectiveness in reducing *Escherichia coli* O157:H7 populations during spray-chilling of inoculated beef carcass tissue. Samples (10 × 20 × 2.5 cm) of beef carcass tissue were inoculated (10^3 to 10^4 CFU/cm^2) with either nonacid-habituated (obtained by incubating at 15°C for 48 h in filter-sterilized composite [1:1] hot and cold water meat washings – W; pH 6.05) or acid-habituated (prepared in W washings mixed with filter-sterilized 2% lactic acid washings in a proportion of 1/99 [vol/vol] – LA/W; pH 4.12) *E. coli* O157:H7 and exposed to conditions simulating carcass chilling (-3°C for 10 h followed by 38 h at 1°C). Spraying treatments applied to samples during chilling included: (a) no spray (NT), (b) water (W), (c) cetylpyridinium chloride (CPC; 0.1 %), (d) ammonium hydroxide (AH; 0.05%) and (e) lactic acid (LA; 2%). Samples were taken at 0, 10, 24, 36 and 48 h of chilling to determine changes in *E. coli* O157:H7 population densities. Overall, spray-chilling was more effective than nonspray-chilling in reducing *E. coli* O157:H7 populations. Regardless of type of inoculum, the effectiveness of the spraying solutions in reducing the pathogen decreased in the following order: CPC > LA > AH > W. Acid-habituated cells were more resistant to LA and CPC interventions than nonacid-habituated cells. Although spray-chilling with antimicrobials may enhance pathogen reduction, previous exposure of *E. coli* O157:H7 to acidic environments may lead to cross-protection from subsequent low pH spray-chilling interventions like LA (pH 2.14) and CPC (pH 4.12).

P029 Thermal Inactivation of *Enterobacter sakazakii* in Rehydrated Infant Formula

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The presence of low levels of *Enterobacter sakazakii* in dried infant formula have been linked to outbreaks of meningitis and necrotizing enterocolitis in neonates, particularly those who are premature or immunocompromised. In the current study the ability of twelve strains of *E. sakazakii* to survive heating in rehydrated infant formula was determined at 58°C using a submerged coil apparatus. The observed D58-values ranged from 30.5 to 591.9 s with heat resistance appearing to be phenotypically bimodal. The z-value of the most heat resistant strain was 5.6°C. When dried infant formula containing this strain was rehydrated with water pre-equilibrated to various temperatures, a greater than or equal to 4-log reduction in *E. sakazakii* levels was achieved by preparing the formula with water above or at 70°C.

P030 Bioluminescent Monitoring of LEE Gene Expression in Living Cells

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Enterohemorrhagic *Escherichia coli* cause a characteristic histopathology in intestinal cells known as attaching and effacing lesion. The genes involved in formation of the AE lesion are encoded by a pathogenicity island named the Locus of Enterocyte Effacement (LEE). Forty-one ORFs were revealed by DNA sequence analysis, and the majority of these genes are organized in five main polycistronic operons, LEE1, LEE2, LEE3, LEE4, and tir. LEE1, LEE2, and LEE3 encode approximately 20 proteins responsible for the type III secretion system. A variety of diverse gram-negative pathogens use type III secretion for translocation of pathogenicity proteins into the cytosol of eukaryotic cells. Using lux reporter gene fusions, expression of operons LEE1, LEE2, and stx_2, shiga toxin gene was continually monitored in living cells. The results showed that although the expression of LEE1 synchronized with cell growth, gene expression lagged cell growth by about 6 to 7 h. In contrast, LEE2 was expressed continually throughout the growth phase. The stx_2 gene was slightly expressed under the conditions used in this work. LEE2 was expressed almost equally in three different *E. coli* hosts, non-toxigenic and toxigenic O157:H7 and an avirulent *E. coli* DH5a. In contrast, LEE1 can only be expressed in the two virulent hosts. Although stx_2 was only expressed at low levels under the conditions we used, it seemed that its expression was independent of the host. Use of this system coupled with in vivo imaging of bioluminescence may enable gene expression to be monitored in live animals.

P031 Survivability of Calicivirus in Foods and on Surfaces: Experiments with Feline Calicivirus as a Surrogate for Norwalk Virus

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While there is sufficient evidence incriminating foods as vehicles for Norwalk-like virus-NLV-(Norovirus) transmission, little is known about virus survival rates in foods and on surfaces. We used feline calicivirus (FCV) as a surrogate for NLV to investigate virus survivability in representative foods of plant and animal origin, and on metal surfaces. 10 µL volumes of a known concentration of FCV suspension were deposited onto 1-cm (diameter) disks of lettuce, strawberries, ham and stainless steel metal, which were place in separate Petri dishes. After air-drying, similar batches of the inoculated items were incubated at 4°C and at 22.5°C. Samples of each item were retrieved from day 1 to day 8, at one-day intervals, and the virus was recovered and plaque assayed. Results from items stored at 4°C showed that 2.5% of the virus was detectable in ham, 0.75% (lettuce) and 0.44% on metal disks on day 6, as compared to 0.7% in strawberries on day 4. At 22.5°C incubation, 0.37% of the virus was detectable on metal disks on day 6, as compared to 0.78% in ham (day 5) and 0.4% in lettuce (day 4). Virus detection in strawberries was inconclusive due to interference by resident microflora. In conclusion, this pilot study indicates that sufficient infectious virus units survived up to 6 days in/on different food and surface matrices, providing valuable information useful for epidemiological and monitoring purposes, and in adopting appropriate food processing practices and measures to control NLV transmission via foods and surfaces.

P032 DSC Survival and Growth of Acid-adapted *Shigella flexneri* in a Traditional Fermented Ghanaian Weaning Food

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Shigallae are among the major causes of diarrheal diseases in infants and young children in developing countries. We conducted a study to determine the effect of fermentation of corn and corn/cowpea doughs used to prepare a traditional weaning porridge on the survival and growth characteristics of acid-adapted and unadapted *Shigella flexneri*. Porridges were prepared from doughs fermented for 0, 24, and 48 h at 30°C. Four-strain mixtures of acid-adapted and unadapted *S. flexneri* cells were separately inoculated (10^4 – 10^5 and 10^6 – 10^7 CFU/ml) into porridges made from unfermented (pH 5.74 – 6.05) and fermented (pH 4.07 – 4.38) doughs. The pathogen survived at high numbers for 24 h, but did not grow, at 10°C and 30°C in porridges made from fermented doughs, while cells inoculated into porridges made from unfermented dough grew at 30°C. Cells inoculated into the porridges containing fermented dough were not detected (<1 CFU/ml) within 4 h at 48°C. Acid-adapted cells survived in higher numbers in porridges made from unfermented dough than did unadapted cells, indicating that prior exposure of cells to mild stress renders them more resistant to subsequent acidic conditions. The addition of cowpea flour to corn dough, followed by fermentation, had little effect on the survival of *S. flexneri* in

porridges made from the dough. Traditional corn-based fermented Ghanaian weaning food can serve as a vehicle for infant shigellosis. It is recommended that porridge not consumed immediately after preparation be boiled before feeding to infants.

P033 DSC Impact of Selected Environmental Stresses on the Resistance of *Listeria monocytogenes* Scott A to Electron Beam Irradiation

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The irradiation resistance of *Listeria monocytogenes* Scott A serotype 4b following starvation, heat shock (40 to 48°C), exposure to low pH (HCl; pH 4.0 to 5.5), high pH (NaOH; pH 8.0 to 11.0) and hydrogen peroxide (H_2O_2; 50 to 500 ppm) was evaluated. Exponential-phase cells of *L. monocytogenes*, grown aerobically at 35°C in tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), were harvested by centrifugation (10,000 times g, 10 min, 4°C), washed in 0.85% (w/v) NaCl then starved for 8 days in 0.85% NaCl (25°C). Exponential-phase cells in TSBYE (35°C) were subjected to heat shock, HCl, NaOH, and H_2O_2 for 1 h. Controls (exponential-phase) were grown aerobically in TSBYE (35°C). All stressed cells and controls were washed in 0.85% NaCl and irradiated in fresh 0.85% NaCl (4°C) at doses ranging from 0.0 to 1.0 kGy. *L. monocytogenes* survivors were enumerated by plating samples on tryptic soy agar with 0.6% yeast extract (TSAYE) and counting bacterial colonies on TSAYE after 72 h at 35°C. The irradiation D value for controls was 0.09 kGy. All stresses, except for H_2O_2 and NaOH, significantly increased the irradiation resistance of *L. monocytogenes*. Starved cells exhibited the highest irradiation resistance with a D value of 0.18 kGy ($P < 0.05$). Cells exposed to 48°C, pH 5.0, and pH 4.5, yielded D values of 0.15, 0.15, and 0.12 kGy, respectively ($P < 0.05$). These results demonstrate that certain stresses cross-protect *L. monocytogenes* against radiation inactivation and should be taken into account when determining the irradiation D-value for this pathogen.

P034 Studies on Enterotoxin Producing *Staphylococcus aureus* Isolated from Dairy Products in Jordan

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A total of 650 dairy samples were collected in northern and middle provinces of Jordan and analyzed for the presence of *Staphylococcus aureus*. Isolates were examined for their biotyping, entero-toxigenicity and thermonuclease activity. A total of 292 *S. aureus* strains were isolated. From milk, ice cream, cheese and jameed dairy samples it was isolated from 50.6%, 33.6%, 52.5% and 9% of the samples, respectively. The mean count obtained ranged from 3.6 to 5.3 $\log_{10}$/ml/g. Biotyping of *S. aureus* strains resulted in determination of human A, human â+ A, poultry B, bovine C and ovine C host specific biotypes and in determination of K-â +CV:C (D), K+â -CV:A, K+â+CV:A and K-â -CV:C, G and F non-host-specific biotypes. A total of 43 strains were found entero-toxigenic and 22 strains were thermonuclease producers. Of those, 27 and 15 strains, respectively, were attributed to host specific biotypes. The examined dairy products were concluded to be hazardous foci of staphylococcal food poisoning outbreaks in Jordan.

P035 Growth of Heat Treated *Clostridium perfringens* Carrying the Enterotoxin Genes on the Chromosome vs. a Plasmid

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Many spores of *C. perfringens* are capable of surviving normal cooking treatment. Only a small proportion of the *C. perfringens*, however, are enterotoxin positive (cpe+). Even more specifically, the strains implicated in human foodborne cases seem to have more heat resistant spores and to carry the enterotoxin genes on the chromosome while strains associated with non-human or non-foodborne disease carry the gene on a plasmid. Little is known of the impact of the two carrier systems on the subsequent germination and growth potential of *C. perfringens*. The objective of the study was to determine the growth potential after heat treatment of two cpe+ isolates with the encoding genes on the chromosome (790–94) and on a plasmid (44071.C05), respectively, at different temperatures and cooling rates in turkey meat. Heat inactivation experiments were conducted using an immersed coil apparatus. Spore germination, out-growth and lag phase, together called GOL time, as well as generation times were determined during constant temperatures in fluid medium as well as in vacuum packed, heat treated minced turkey. GOL time and growth were also followed during cooling scenarios over a period of 3, 4, 5, and 6 h in minced turkey. Spores of strain 790–94 were approx. 10-fold more heat resistant at 85°C than those of strain 44071.C05. Furthermore 790–94 had a higher temperature growth range plus shorter GOL times and approx. half the generation time compared with 44071.C05 at temperatures of > 45°C

in turkey meat. 790–94 increased by 0.4, 1.0 and 1.6 log, respectively, during cooling from 65°C to 10°C in 4, 5, and 6 h.

P036 DSC Expression of Cold Shock Proteins by *Yersinia enterocolitica* in Synthetic Medium and Foods

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Yersinia enterocolitica is a psychrotrophic foodborne pathogen, and has been implicated in outbreaks involving cold-stored foods, especially milk and pork. To develop effective strategies to prevent growth of microorganisms at low temperatures, it is critical to understand the mechanisms involved in cold tolerance. A major adaptation mechanism of bacteria to cold is expression of cold shock proteins. The objective of this research is to study the expression of major cold shock proteins of *Y. enterocolitica* in Luria-bertani (LB) medium, milk and pork following a temperature downshift from 30 to 4°C. *Y. enterocolitica* (10^9 CFU) was inoculated into 10 ml of LB or sterile skim milk or pork loins, and the samples were stored at 4°C (cold shock) or 30°C (control) for 0, 4, 8, and 12 h. At each sampling time, *Y. enterocolitica* harvested from LB, milk and pork were disrupted by agitation with 0.1mm Zirconia beads. The cell lysate was collected, and extracted total protein was subjected to 2-D gel electrophoresis. Two proteins (CspA1/CspA2) of ~7 Kda were expressed by *Y. enterocolitica* following cold shock in LB, milk, and pork. However, CspA1 and CspA2 were not expressed by *Y. enterocolitica* at 30°C. CspA1 and CspA2 were observed as early as after 4 h of cold shock in *Y. enterocolitica* in LB and milk, whereas expression of the proteins was found only at 12 h of cold shock in *Y. enterocolitica* from pork. This study is the first report on expression of cold shock proteins by a psychrotrophic bacterium in foods.

P037 Effects of Hot Water and Lactic Acid Applied Singly and in Combination on Survival and Growth of *Salmonella* on Fresh Beef Stored at 4, 10 or 25°C

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This study evaluated survival or growth of *Salmonella* during storage of fresh beef subjected to different decontamination treatments. A five-strain mixture of *S.* Typhimurium was inoculated onto fresh beef pieces (2.5 × 5 × 1 cm) and then immersed (30 s) in hot water (HW; 75°C), 2% lactic acid (LA; 55°C), hot water followed by lactic acid (HW/LA), or lactic acid followed by hot water (LA/HW). Samples were tray-overwrapped with air-permeable film and stored at 4, 10 or 25°C for 15, 10 or 5 days, respectively. Microbial populations were determined on tryptic soy and XLD agar. Initial populations (5.19 log CFU/cm²) of *Salmonella* were reduced by 1.92 (LA) to 3.00 (HW/LA) log CFU/cm² immediately after application of treatments, with combination treatments resulting in higher reductions (2.77–3.00 log CFU/cm²) than single treatments (1.92–2.04 log CFU/cm²). Numbers increased to > 6.98 log CFU/cm² after 3 days of storage at 25°C for all treatments except HW/LA, where levels remained 2.80 log lower than the control (C) even after 5 days. After 10 days at 10°C, populations for LA and HW/LA samples were 3.03 and 4.90 log CFU/cm² lower, respectively, than C samples. In comparison, LA/HW samples were 3.55 log CFU/cm² higher than HW/LA samples at the end of the 10°C storage period. *Salmonella* populations changed by 0.02 to -0.80 log CFU/cm² on samples of all treatments stored at 4°C. Results indicated that survival or growth of *Salmonella* during storage may be affected by decontamination treatment, as well as its sequence of application, and this should be considered when implementing decontamination intervention strategies.

P038 Molecular Surveillance of Shiga Toxigenic *Escherichia coli* O157:H7 by PulseNet USA in 2002

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The PulseNet Network, established in the United States by the Centers for Disease Control and Prevention in 1996, has proven its utility for rapid recognition of foodborne disease clusters, and has facilitated accelerated investigation of outbreaks. PulseNet USA handles laboratory information in three ways: (1) It acts on information posted by participating laboratories on the Web-based rapid alert system (The PulseNet WebBoard); (2) It continuously evaluates and compares the pulsed-field gel electrophoresis (PFGE) patterns of clinical isolate patterns submitted to the PulseNet database at the CDC to detect disease clusters as early as possible; and (3) It initiates a follow-up investigation whenever a food isolate pattern submitted by a food regulatory agency matches the PFGE pattern of a human isolate submitted to PulseNet within the same time frame. In 2002, 38 inquiries about *Escherichia coli* O157:H7 were posted on the WebBoard by the participating public health laboratories. Thirty-four of these represented local

outbreaks, and four were multi-state outbreaks. Patterns for 3,392 *E. coli* O157:H7 isolates were submitted to the PulseNet database in 2002. Forty-one clusters that were detected only by routine PulseNet pattern comparisons were further investigated; seven of these were determined to be common source outbreaks. In five outbreaks, one or more *E. coli* O157 isolates with a PFGE-pattern indistinguishable from the corresponding outbreak pattern were found in the implicated food. PulseNet is an efficient early warning system for detecting foodborne disease clusters and a critical component of foodborne disease outbreak investigations.

P039 Laboratory Investigation of a Multistate Outbreak of Listeriosis in the Northeastern United States, 2002

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Listeriosis is a foodborne disease that has devastating consequences for the fetus, the newborn, and immunosuppressed adults. An estimated 2,500 cases of sporadic *Listeria* infections occur in the United States each year and 20–25% of the infected persons die. During the summer of 2002, a significant increase in the number of listeriosis cases occurred in the northeastern United States. Characterization of clinical isolates using pulsed-field gel electrophoresis (PFGE) by public health laboratories participating in CDC's PulseNet network indicated that 29% of 186 listeriosis patients from nine states were infected by the outbreak strain, defined as yielding the same PFGE pattern (PulseNet pattern numbers: GX6A16.0235 and GX6A12.0003) after restriction of genomic DNA with AscI and ApaI enzymes. The outbreak strain represented < 1% of isolate patterns in the PulseNet National Database from 1995 through June 2002. Real-time serotyping, ribotyping and PFGE typing enabled epidemiologists to distinguish outbreak-associated cases from other geographical and temporal-associated cases and to link outbreak-associated infections with the consumption of contaminated turkey-based deli products. The outbreak strain was recovered from the environment of one deli turkey meat manufacturing facility and from the intact product of another facility. Timely and routine subtyping of clinical isolates of *Listeria monocytogenes* and submission of subtype data to the national PulseNet database by participating public health laboratories are essential for rapid recognition of disease clusters and prompt deployment of epidemiologic resources for investigations.

P040 School-related Foodborne Disease Outbreaks in the United States

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Each year millions of American children eat meals prepared at school. Recent media reports suggest an increased risk of foodborne illness associated with meals prepared at schools. We reviewed data on foodborne outbreaks reported through the National Foodborne Outbreak Reporting System (FORS) from 1973 through 2000. A school outbreak was defined as two or more ill persons ingesting a common food prepared in that school. From 1973 to 1997, 488 (3.7%) of 13,173 reported foodborne outbreaks involved food prepared in schools. From 1998 to 2000, 128 (3%) of 4069 reported foodborne outbreaks involved food prepared in schools. Reported outbreaks in schools from 1998 to 2000 accounted for 7993 (10%) illnesses, 119 hospitalizations (6%), and no deaths. The median number of estimated cases per outbreak from 1998 to 2000 was 41 persons (range 2 to 644). An etiology was confirmed in 51 (40%) of 128 school-related outbreaks; among outbreaks with confirmed etiology, 41% were caused by Norwalk-like virus. In most (66%) of school-related outbreaks, a particular food item was not implicated. Expressed as a proportion of outbreaks reported to CDC, the percentage of outbreaks from foods prepared at schools has not increased in recent years. Nevertheless, efforts to ensure the safe production, storage, and preparation of food in schools should remain a priority. Improvements in the capacity to investigate foodborne outbreaks may be needed to identify the proportion of outbreaks in which a food item is implicated. Further studies should address specific food items identified during outbreaks and preventative measures needed to preclude these outbreaks.

P041 Contributing Factors to Foodborne Disease Outbreaks: Lessons Learned from the Foodborne Outbreak Reporting System, 1998–2000

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Contributing factors (CFs) are defined as practices or conditions that may contribute to the contamination of a food item, and thus to foodborne illness. We analyzed data on CFs associated with outbreaks reported by state health departments to CDC's Foodborne Outbreak Reporting System (FORS), from 1998 through 2000. Thirty-

seven types of CFs were reportable on the Foodborne Outbreak Reporting form. CFs were classified into the following categories for analysis purposes: pre-harvest, post-harvest, contamination, ill foodhandler, storage/holding, or cooking. If no factors were reported, the CFs were classified as unknown. CFs were provided for 1706 (42%) of 4069 reported outbreaks. Among confirmed *E. coli* O157:H7 (O157) outbreaks, 31 (48%) reported at least one CF: 10 (15%) reported inadequate/improper cooking, 9 (14%) reported contamination, 6 (9%) reported pre-harvest contamination, 3 (5%) reported an ill foodhandler and 3 (5%) reported improper storage/holding. Among confirmed *Salmonella* Enteritidis (SE) outbreaks, 84 (65%) reported at least one CF: 46 (35%) reported inadequate/improper cooking, 23 (17%) reported improper storage/holding, 8 (6%) reported pre-harvest contamination, 5 (4%) reported contamination, and 2 (2%) reported an ill foodhandler. Reported CFs for confirmed SE and O157 outbreaks illustrate the increased need for foodhandler training in proper cooking, handling, and storage procedures. In addition, the amount of pre-harvest contamination needs to be reduced. Local and state epidemiologists and sanitarians need additional training to investigate factors contributing to foodborne disease outbreaks.

P042 Foodborne Disease Outbreaks of Undetermined Etiology, 1998–2000

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Despite the discovery of new pathogens and improvements in laboratory detection methods, many foodborne disease outbreaks in the United States are of undetermined etiology. To better define the epidemiology of Foodborne Outbreaks of Undetermined Etiology (FOUE), we reviewed outbreaks reported to CDC through the Foodborne Outbreak Reporting System for the years 1998 to 2000. We defined a FOUE as one not meeting published criteria for an outbreak of known etiology. FOUEs were categorized into pathogen-specific syndromes defined by J.A. Hall and colleagues. FOUEs accounted for 2,113 (52%) of 4,069 foodborne disease outbreaks reported to CDC for the years 1998 to 2000 and resulted in 26,138 illnesses, 297 hospitalizations, and 10 deaths. Compared with foodborne outbreaks of known etiology, FOUEs had a smaller median size (4 versus 11 persons), were less likely to identify a food vehicle (1,087 [51%] vs 1,364 [70%], RR=0.70, $P < 0.0001$), and were more likely to identify a restaurant as the place of food preparation (1,483 [70%] versus 1,060 [54%], RR=1.4, $P < 0.0001$). FOUEs fell into the following syndromes: Norwalk (Norovirus)-like syndrome (36%), vomiting-toxin syndrome (26%), *Salmonella*-like syndrome (9%), diarrhea-toxin syndrome (2%), and *E. coli* syndrome (<0.5%). FOUEs were generally smaller than outbreaks of known etiology but contributed to the overall morbidity of foodborne illness. Norovirus is probably a leading cause of outbreaks of undetermined etiology but bacterial agents including *Salmonella* and diarrheagenic *E. coli* also contribute. Efforts to improve outbreak investigation, including prompt specimen collection, may decrease the number of outbreaks due to undetermined etiology.

P043 DSC Tracking Canadian Foodborne Outbreaks: A New Tool for Canadian Researchers

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Readily available, centralized information linking the incidence of foodborne disease to the source of infection in Canada is lacking. Therefore we created a database of all known information on Canadian and international foodborne disease outbreaks. This Microsoft Access database contains past outbreak information and is designed so that new outbreak information can be added as it becomes available. The database quickly and easily performs queries and generates reports. Information for each outbreak includes: commodity, etiologic agent, setting, location, date, attack rate, concentration of organism in food if available, source, verified yes or no, and a narrative with a web-link to the original source if available. Information for these outbreaks is compiled from peer-reviewed journals, newspapers, list-servs, press releases, and government websites. Since the majority of foodborne outbreaks go unreported, stories on outbreaks from unverifiable sources are included to draw a wider picture of foodborne outbreaks. Also included are stories on outbreaks where the food and/or etiologic agent were unknown since this is often a reality. The next step will be to engage public health units across Canada by contributing foodborne disease reports. This will make the information more relevant than relying on published reports alone, which may be one to two years behind. Ultimately, we wish to have the entire up-to-date database readily accessible on the Internet for current information dissemination. This tool could then be used in scientific-based decision-making to identify research needs and to develop policy for both the food industry and government.

P044 DSC Quantification of Biofilm Formation by Cold-injured and Cold-starved *Listeria monocytogenes*

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Presence of *Listeria monocytogenes* strains endemic to food processing environments is presumably related to biofilm formation. Following exposure to various environmental stresses, *Listeria* cells may be more prone to attach to surfaces. This study quantified the degree of biofilm formation in a defined set of *L. monocytogenes* strains when uninjured, cold-shocked or starved. Twenty-two *L. monocytogenes* strains (food and dairy plant isolates) were selected from a set of 129 strains previously characterized for biofilm formation. *L. monocytogenes* (10^2 CFU/ml) was either cold-shocked (4°C / 2 h in Modified Welshimer's Broth-MWB), or cold-starved (4°C/10 days in Butterfield's Phosphate Buffer). Uninjured cultures (35°C/24 h on TSA-YE) were used as controls. Biofilm formation by the uninjured, cold-shocked or starved cells was quantified in MWB using 96-well untreated polystyrene microtiter plates (3 wells/strain × 3 replicates). Following 4 days of incubation at 22°C, the microtiter plate wells were emptied, rinsed and air-dried. After staining cells that were fixed in 99% methanol with crystal violet, biofilm formation as measured by optical density (OD) of the resolubilized dye was read spectrophotometrically at 570 nm. Prior injury of *L. monocytogenes* by starvation (16.7% injured) and cold (47.1% injured) enhanced biofilm formation. The mean ODs of cold-shocked, starved and uninjured cultures were 1.456 (range, 0.116–3.730), 1.262 (range, 0.082–3.766) and 0.943 (range, 0.137–2.605), respectively. Strains that comprised the extreme OD values in biofilm formation remained consistent between all three treatments. Dairy plant isolates did not predominate as strong biofilm formers.

P045 Comparative Characterization of Two *Listeria monocytogenes* Isolates That May Have Originated from the Same Strain Persisting in the Same Food Processing Establishment for Over a Decade

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The *Listeria monocytogenes* strain that caused a multistate outbreak of listeriosis in the United States in May 2000 was traced to contaminated deli turkey product produced at a processing facility in Waco, Texas. The same processing facility was identified as the source of contaminated turkey-based hot dogs that caused a fatal case of sporadic listeriosis in 1989. To determine if the same strain of *L. monocytogenes* may have persisted in the food processing facility for 11 years, we characterized the two isolates by PFGE of genomic DNA with multiple restriction enzymes, sequencing of actA and inlA virulence-associated genes, RAPD analysis using four primers, plasmid profile analysis, and restriction fragment length polymorphism (RFLP) analysis using mixed *L. monocytogenes*-specific DNA probes. The two isolates had identical DNA sequences for actA and inlA genes. RAPD analysis and PFGE using restriction enzymes ApaI, AscI, XbaI, and SmaI revealed no differences between the two isolates. However, different PFGE patterns were obtained with use of the restriction enzyme NotI. Also, each isolate harbored a different plasmid. Southern hybridizations of NotI-digested genomic DNA of the two isolates using each plasmid as a probe revealed different hybridization patterns, indicating sequence differences between the plasmids. We conclude the two isolates have only minor genetic differences, possibly caused by the acquisition or loss of plasmid sequences. Our data support the hypothesis that the same strain of *L. monocytogenes* had persisted in the food processing establishment for more than a decade.

P046 Human Infections in Canada Caused by *Listeria monocytogenes*

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The objectives of this study were to compile and summarize information related to human infections caused by *Listeria monocytogenes* in Canada and to characterize the isolates responsible. Isolates of *L. monocytogenes* submitted were characterized by serotyping and pulsed-field gel electrophoresis (PFGE). Additional epidemiological information was solicited, including patient age and sex, site of isolation, and geographical origin. Laboratory-based surveillance detected between 47 and 79 cases of human listeriosis each year from 1995 through 2000, predominantly in individuals 50 years of age or older. Most isolates were cultured from blood or cerebrospinal fluid, though *L. monocytogenes* was also obtained from a number of other sterile sites, as well as from stools. Serotype 1/2a was most commonly found (55%), followed by serotype 4b (23%). Though more than 193 PFGE types were detected, only a few of these isolates

appeared to persist for more than one or two years, or cause more than three or four cases of human disease. However, the predominance of one or two PFGE types in several provinces in one or two years suggests the possibility that smaller outbreaks may have occurred. At present, infections due to *L. monocytogenes* are not nationally reportable. Serotyping, PFGE, and other typing or fingerprinting methods provide valuable information about infections caused by this organism, and may help detect outbreaks and track sources of infection.

P047 DSC Investigation of the Role of Quorum-sensing Mechanisms on Virulence Factor Expression in *Listeria monocytogenes*

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Cell density dependent regulation of gene expression (quorum-sensing) has been demonstrated to modulate such diverse functions as virulence, competence, sporulation and biofilm formation. Quorum-sensing systems in Gram-positive organisms are based on a two-component regulatory system, consisting of a histidine kinase sensor molecule and a response regulator, with cell density monitored via a peptide pheromone. Quorum-sensing has not yet been described in *Listeria monocytogenes*. PCR was used to screen ten *L. monocytogenes* and 12 non-*monocytogenes* isolates for seven response regulators that have recently been identified in a *L. monocytogenes* reference strain. All seven response regulators were ubiquitous in *L. monocytogenes*. Three of the response regulators were unique to *L. monocytogenes*, and, of these, two were previously found to play a role in virulence. Virulence factor expression, monitored via luxAB gene fusions to listeriolysin and phospholipase C, was inducible by a change in media and by heat shock but was not inducible using cell-free culture supernatants of *L. monocytogenes*. SELDI-TOF-MS was used to identify peptides produced by *L. monocytogenes* cultures at high cell-density. Analysis of cell-free lysates in rich media and chemically defined media has not yet resulted in the identification of peptides worthy of further study. To date, studies have been unable to identify molecules involved in quorum-sensing in *L. monocytogenes* and thus its role in virulence remains to be clarified. The response regulators that were found only in *L. monocytogenes* may be involved in stress responses and virulence mechanisms unique to the pathogenic species of *L. monocytogenes*.

P048 Modeling Liquid and Surface Growth Limits of *Listeria monocytogenes* as a Function of pH, a_w and Temperature

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The microstructure of food may affect microbial growth by imposing physical restraints that may limit nutrient diffusion. The objective was to evaluate differences in effects of incubation temperature, pH and a_w on growth of *Listeria monocytogenes* and to model its growth limits in liquid and solid substrates. Growth initiation of a 5-strain mixture of *L. monocytogenes* in tryptic soy broth (TSB) and tryptic soy agar (TSA) was studied in 280 combinations of temperature (4–30°C), pH (3.96–6.44) and a_w (0.900–0.990) adjusted with NaCl. Growth in TSB was detected periodically for 60 days by recording turbidity (620nm) with an automated microplate reader, while on TSA it was observed through initiation of colony formation. Absence of growth was confirmed by plating of broth or agar on TSA and comparing populations with the inoculum size (Student's t-test). Data were modeled using a logistic regression process. The models fitted the data with concordance rates of 99.8 and 99.9% for liquid and surface growth, respectively. A difference between growth limits of *L. monocytogenes* in liquid and solid media was observed. For example, at 25°C and a_w of 0.990 the predicted pH limits of the pathogen in TSB and TSA were 4.38 and 4.74, respectively, while at 10°C and pH 5.5 the a_w limits were 0.912 and 0.958 for liquid and surface growth, respectively. The results indicated that the growth limits of the pathogen were higher in colonial compared to planktonic growth. Thus, in order to predict microbial behavior in foods accurately, it is important to ascertain if the organism exhibits colonial or planktonic growth and use the appropriate model.

P049 DSC Unusual Genetic Features of the *Listeria monocytogenes* Strains Implicated in a Recent Mexican-style Soft Cheese Outbreak

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In 2000 to 2001, *Listeria monocytogenes* serotype 4b was implicated in an outbreak of listeriosis traced to homemade, Mexican-style soft cheese produced from contaminated raw milk in Winston-Salem, North Carolina (NC). Molecular markers specific for these strains have not yet been identified. In this study, these clinical isolates have revealed an unusual combination of genetic markers, which include a restriction fragment length

polymorphism (RFLP) pattern typical of strains implicated in several previous outbreaks in Europe and North America, belonging to "Epidemic Clone I" (ECI). However, unlike ECI strains, the genomic DNA of the NC strains was sensitive to digestion by the restriction enzyme Sau3AI, suggesting a lack of DNA methylation at the cytosine of GATC sites. Additionally, Polymerase Chain Reactions and Southern blots indicate an unusual distribution of several genomic regions that have recently been found to be unique to ECI but absent from other serotype 4b strains. The genome of NC strains harbors one of these regions but lacks several others, including the genes involved in cytosine methylation. Southern blots hybridized with probes derived from the shared regions showed that highly conserved sequences were present only in ECI, NC, and serotype 1/2b strains. In contrast, other strains of serotype 4b appeared to have undergone significant divergence in this region. These findings suggest that the NC strains have some, but not all, of the unique genetic attributes of ECI. The evolutionary mechanisms responsible for the unusual genetic markers in these strains and their possible impact on virulence remain unclear.

P050 Thermal Resistance of *Listeria monocytogenes* Scott A during Starvation in Phosphate Buffer, 0.85% Sodium Chloride, or Phosphate Buffered Saline

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A study was conducted to determine the thermal resistance of *Listeria monocytogenes* Scott A during starvation in phosphate buffer (PB), 0.85% (wt/vol) sodium chloride (NaCl) or phosphate buffered saline (PBS). PB, 0.85% NaCl (wt/vol) or PBS, inoculated with washed cells (10^7 CFU/ml) from a stationary-phase culture (20 h, 35°C) of *L. monocytogenes* Scott A, was held at 30°C for 7 days. Viability of the organism and its heat resistance were determined at daily intervals during starvation. Cells were harvested by centrifugation (10,000 × g, 10 min, 4°C), washed in PB, and heated (60°C) in fresh PB. Numbers of viable cells in each medium declined by approximately 1.5 log after the first day of starvation and remained relatively constant for the remaining six days. The D-value of *L. monocytogenes* Scott A prior to starvation (day 0) was 0.5 min. Thermal resistance of *L. monocytogenes* Scott A, starved in PB and PBS, increased steadily and reached D-values of 5.0 and 5.2 min, respectively, at day 7. In contrast, starvation of the organism in 0.85% NaCl (wt/vol) consistently produced a minimal increase in thermal resistance; the D-value at day 7 was 0.65 min. These results indicate that the type of medium used to starve *L. monocytogenes* can affect its heat resistance and should be taken into account when evaluating the D-value of this pathogen following starvation.

P051 Survival and Recovery of Viable but Non-Culturable (VBNC) *Listeria monocytogenes* Cells Starved in a Nutritionally Depleted Medium

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Survival of a five-strain *Listeria monocytogenes* culture, including serotype 4b, during starvation in sand at 4°C for 2 months was determined using the acridine orange direct count (AODC) method and plate count. Sand samples inoculated with *L. monocytogenes* were taken every two weeks. The inoculated sand was then subjected to a 6-h incubation at 37°C and fixed with acridine orange. Elongated VBNC cells were observed more often during weeks 2 and 4 under a fluorescent microscope. At weeks 6 and 8, some of the cells either remained viable or were dead. In each microscopic field, only one or two VBNC cells were observed with hundreds of other viable culturable cells, indicating that *L. monocytogenes* does not readily become VBNC; the bacterium is either viable and culturable or dead. Therefore, when plating environmental samples or stressed *L. monocytogenes* cells on non-selective media, VBNC cells are not a significant concern. Media used for plate count included tryptic soy agar with 0.6% yeast extract (TSBYE) and Columbia agar as non-selective media and Modified Oxford agar (MOX) as the selective medium. Variations within these media included aerobic or anaerobic incubation, with 0.1% or 1% sodium pyruvate to determine the best condition to recover the highest number of injured cells. Results from the non-selective media showed better recovery observed on TSBYE incubated aerobically and with 0.1% pyruvate and Columbia agar incubated aerobically and with 0.1% pyruvate. However, these methods were statistically indistinguishable from each other ($P > 0.05$).

P052 Evaluation of Nisin-Coated Cellulose Casings for Control of *Listeria monocytogenes* on the Surface of Frankfurters Formulated with Lactates and Stored At 4°C

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Biopreservatives such as nisin and organic acids such as potassium lactate and sodium diacetate have proven to be effective in the control of *Listeria*

monocytogenes (Lm) in various foods including RTE meats. The present study was conducted to determine if frankfurters formulated with 1.32% potassium lactate and 0.09% sodium diacetate that were subsequently processed in cellulose casings coated with and without nisin (ca. 50,000 I.U. per square inch of surface area) would control the outgrowth of Lm during refrigerated storage. Packages containing the frankfurters were inoculated with approximately 5 $\log_{10}$ CFU per package of a five-strain mixture of Lm and stored at 4°C for 45 days. Survivors were recovered and enumerated by rinsing each package with sterile peptone water and direct plating onto MOX selective agar. The data for each of two trials were averaged. In packages containing frankfurters prepared in nisin-coated casings, Lm levels decreased by about 1.1 $\log_{10}$ CFU per package after 45 days of storage. In comparison, Lm levels decreased by about 0.6 $\log_{10}$ CFU per package in frankfurters prepared in casings that were not coated with nisin. In a related study, using the same nisin-coated casings and frankfurter formulation, similar results were obtained using a different three-strain mixture of Lm strains, demonstrating that these antimicrobials are effective against a variety of strains. These data establish that potassium lactate and sodium diacetate used alone and in combination with nisin-coated casings can enhance the safety of frankfurters by controlling the levels of Lm during storage at 4°C.

P053 Effects of Irradiation on Survival and Growth of *Listeria monocytogenes* and Natural Microflora in Vacuum-packaged Turkey Ham

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The survival and growth of *Listeria monocytogenes* and natural microflora in turkey ham were evaluated in response to electron beam irradiation. Numbers of naturally-occurring bacteria on sliced turkey ham were 5.2×10^2 CFU/cm^2. Slices of ham were inoculated with a five-strain mixture of *L. monocytogenes* to obtain ~ 3.0×10^6 CFU/cm^2, vacuum packaged, and irradiated at 0 (control) to 2.5 kGy. Ham irradiated at 0, 1.0, and 2.0 kGy were stored at 4°C for 28 days. *L. monocytogenes* (from inoculated ham) and aerobic plate count (APC) (non-inoculated ham) were determined by plating diluted samples on Modified Oxford (MOX) agar and tryptic soy agar with 0.6% yeast extract (TSAYE), respectively. Bacterial colonies on inoculated agar plates were counted after incubation (30°C, 48 h). The D-value of *L. monocytogenes* was 0.52 kGy. $\log_{10}$ reductions of *L. monocytogenes* following irradiation at 1.0 to 2.5 kGy ranged from 2.0 to 5.5. APC was reduced to < 10 CFU/cm^2 at 2.0 kGy. In 2.0 kGy-treated ham at 4°C, *L. monocytogenes* grew to 2.58 and 4.82 $\log_{10}$ CFU/cm^2 at 14 and 18 days, respectively; APC increased to 1.06 and 2.98 $\log_{10}$ CFU/cm^2, respectively. Irradiation (1.0 to 2.5 kGy) reduced *L. monocytogenes* and APC in turkey ham. From the D-value obtained in this study, at least 2.6 kGy could achieve a 5-log reduction of *L. monocytogenes* in turkey ham. Additional barriers to growth of *L. monocytogenes* in turkey ham may be necessary to ensure the microbial safety of this product following low-dose irradiation.

P054 DSC Persistence of *Escherichia coli* O157:H7, *Salmonella* Newport, and *Salmonella* Poona in the Gut of a Free-living Nematode, *Caenorhabditis elegans*

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Caenorhabditis elegans, a free-living nematode found in soil, has been shown to ingest human enteric pathogens, thereby potentially serving as a vector for preharvest contamination of fruits and vegetables. A study was done to determine the persistence of *Escherichia coli* O157:H7 and *Salmonella* in the gut of *C. elegans* as affected by temperature. Adult worms were fed nalidixic acid-adapted cells of *E. coli* OP50 (control), two strains of *E. coli* O157:H7, *Salmonella* Newport, or *Salmonella* Poona for 3 h, washed, placed on K agar with a lawn of *E. coli* OP50 not adapted to nalidixic acid or on uninoculated Bacto agar, and incubated at 20°C or at 4, 20, and 37°C, respectively, for up to 5 days. Populations of pathogens (CFU/worm) were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic acid. Initial populations of pathogens (2.5 – 3.2 log CFU/worm) significantly ($P \leq 0.05$) increased by up to 2.9 log within 1 day and remained constant for an additional 4 days on K agar incubated at 20°C. Compared to day 0, populations of test pathogens did not differ by more than 1 log CFU/worm when worms were incubated on Bacto agar at 4°C for up to 3 days; at 20°C, populations decreased significantly by day 3. Populations recovered from worms incubated at 37°C significantly increased within 1 day. Results indicate that the length of time *E. coli* O157:H7 and *Salmonella* persist in the gut of *C. elegans* is greatly affected by temperature.

P055 Incorporation of Sodium Lactate and/or Sodium Diacetate Enhances Thermal Destruction of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, or *Listeria monocytogenes* in Meat and Poultry Products

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Several generally regarded as safe (GRAS) food additives are known to improve the microbial safety of fresh and ready-to-eat meat and poultry products. In this study, GRAS compounds were used in combination with thermal processing to inhibit *E. coli* O157:H7, *Salmonella* spp., or *L. monocytogenes* in beef/pork, all beef, or turkey meatballs, or marinated wafer steaks. Bestate DS-2 (consisting of 54 to 57% sodium lactate, 4 to 5% sodium diacetate) or Vinlac DS (consisting of 49 to 53% sodium lactate, 47 to 51% vinegar) were added to beef/pork meatballs at a concentration of 2% and inoculated with a cocktail consisting of > 6 $\log_{10}$ CFU/ml of two strains of each of the three pathogens. The pathogens were either enumerated or analyzed for the presence/absence in raw products before cooking (4°C, control) or following cooking to an internal temperature of 66, 69, or 71°C (±2°C), and again after 30 days of frozen storage at -20°C. Preliminary results indicated that pathogen populations associated with beef/pork meatballs containing Vinlac DS were reduced to a greater extent than product containing Bestate DS-2. Subsequent studies demonstrated that a combination of 2.5% Vinlac DS and thermal processing to an internal temperature of 69 to 71°C (±2°C) reduced pathogen populations > 6 $\log_{10}$ CFU/g in beef and turkey meatballs, as well as marinated wafer steaks. The results also demonstrated that the addition of 2.5% Vinlac DS enhanced destruction of the microbes such that the final product internal temperature could be reduced, thereby saving cooking time and thermal energy, and reducing product weight loss during the cooking process.

P056 Control of *Campylobacter jejuni* on the Surface of Raw Chicken Coated with Edible Zein Films Containing Ethylenediaminetetraacetate and/or Nisin

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We determined the inhibitory activities of ethylenediaminetetraacetic salt (EDTA) with or without zein edible films containing nisin coated onto the surfaces of raw chicken for protection against *Campylobacter jejuni* (CJ). Raw chicken breast samples (5 g) were immersed into 48 h diluted broth cultures of CJ for 1 min and then allowed to drip free of excess inoculum. The meat samples were dipped into an edible zein film dissolved into propylene glycol (ZP), with and without added nisin (N) (1000 IU/g) and/or EDTA (1.6 mg/ml). Samples were placed into sterile bags, refrigerated at 4°C for up to 28 days. Bacterial counts were examined at 0, 7, 14, 21, and 28 days by spread plating on Bolton agar with antibiotic supplement and 5% lysed horse blood and then incubating under microaerophilic conditions at 42°C for 48 h. For controls, an initial 7.5 log CFU/g inoculum of CJ on the surface of chicken dropped to about 4 log CFU/g at day 14 through 28 at 4°C. EDTA alone and ZPN reduced CJ counts to non-detectable levels on the surface of raw chicken from 14 to 28 days. The ZP + EDTA and ZPN + EDTA coatings reduced CJ counts to non-detectable from 7 to 28 days. The CJ counts on raw chicken samples coated with N or ZP were not significantly different from the controls counts throughout the 28 days. Our results indicated that zein films with antimicrobial agents and/or EDTA show promise for the control of *Campylobacter* on the surface of raw chicken.

P057 Comparison of Attachment and Penetration Abilities of *Campylobacter jejuni* Isolated from Humans and from Chicken Carcasses Acquired at Processing and Retail

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Campylobacter jejuni has emerged as an important foodborne pathogen. *C. jejuni* causes a variety of symptoms and has been linked to foodborne illnesses related to poultry. The ability of *C. jejuni* to adhere to and penetrate intestinal cells has been shown to be a primary factor associated with pathogenicity and is the focus of this experiment. To compare the adherence and penetration ability of *C. jejuni* isolated from chicken carcasses to that of *C. jejuni* isolated from humans, 100 *C. jejuni* were isolated, 25 from pre-chilled chicken carcasses, 25 from post-chilled chicken carcasses, 25 from retail chickens, and 25 from humans exhibiting symptoms of campylobacteriosis. Using INT 407 human intestinal cells as the in vitro model, overall ability to attach to and penetrate into human intestinal cells was determined for each isolate within each category. While wide variation was found for both pathogenic factors within each group of isolates, the human isolates appear to possess a greater ability to penetrate into human

intestinal cells. This indicates that campylobacteriosis may be more highly dependent upon penetration ability as a pathogenicity factor than upon attachment alone.

P058 Evaluation of Coliform and *Escherichia coli* Methods for Testing Raw Materials and Finished Products of Nutritional Foods and Supplements

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It was noted that the ColiComplete method (AOAC Official Method 992.30) used in-house for coliform and *E. coli* testing has limitations with some of the matrices. Such limitations have added labor and time costs to the QA Labs because of the additional confirmatory tests required. The objective of this study was to compare various coliform and *E. coli* methods with the ColiComplete method for enumeration of coliforms and *E. coli* in food samples. The Violet Red Bile agar (VRBA) with MUG and two rapid methods, the SimPlate for Total Coliform and *E. coli* Color Indicator (CEcCI), Petrifilm *E. coli*/Coliform Count (EC) Plate, were evaluated against the ColiComplete method for 11 raw materials/finished products. These samples included nutritional bars and drink mixes, and natural raw materials such as parsley, spinach, and alfalfa. Of the 11 samples tested, 7 were artificially inoculated and 4 were naturally contaminated. Plate counts using VRBA with MUG and Petrifilm EC plate produced comparable results. Overall, the SimPlate method produced consistently higher counts than the VRBA with MUG and Petrifilm methods, and greater variability between duplicates of the same sample. Counts from the ColiComplete generally had greater variability between triplicate samples than the other methods. This was most likely due to counts obtained from the MPN table rather than the actual counts. The Petrifilm EC plate gave more accurate and less variable results for the artificially inoculated food products tested; however, it was not suitable for samples containing high particulate matter such as the natural raw materials.

P059 Study on Presumptive Test Methods for the Detection of *Escherichia coli* in High-salted Seasoning

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Presumptive detection of *E. coli* in high-salt seasoning often fails because of the growth inhibition caused by high salt contents. A study comparing presumptive test methods was performed to determine the detection limit of *E. coli* in food. An *E. coli* (ATCC 25922) culture was diluted to final concentrations of 10^0, 10^1, 10^2, 10^3, 10^4, and 10^5 CFU/ml and these serially diluted cultures were inoculated into a variety of food samples, including high-salt seasoning. Challenged food samples were inoculated into tubes in triplicate containing EC medium, EC-MUG (4-methylumbelliferyl-ß-D-glucuronide) medium, Lauryl Tryptose broth, and Lauryl Tryptose broth-MUG, and incubated at two temperature conditions (at 35°C and 44.5°C). The test method using Lauryl Tryptose broth-MUG at 35°C is most sensitive and rapid for the detection of *E. coli* in high-salt seasoning; the presence of two viable cells was detected within 24 h. Cells injured by high salt content were effectively recovered and easily detected in the Lauryl Tryptose broth at 35°C.

P060 Validation of a New ELISA-based Method for the Detection of VTEC in Food

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Verocytotoxin-producing *E. coli* (VTEC), including *E. coli* O157:H7, the first described serovar of importance, have been implicated in various foodborne outbreaks since 1982. Several other serovars, such as *E. coli* O:26, O:103, O:111 and O:145, were recently implicated in outbreaks worldwide. Today, the cultural methods specific for the pathogenic serovars allow mainly the detection of *E. coli* O157 and so cannot be used routinely for the other serovars. In order to screen for the presence of all these VTEC in food, we have developed a method combining a first step enrichment mTSBn (16–21 h) and an ELISA specific for VT1 and VT2. This 4-step sandwich type ELISA was demonstrated to be specific for VT1 and VT2 produced by *E. coli* strains (n = 16) and did not show any cross-reactivity towards other toxins, non-VTEC strains and other bacteria belonging to the Enterobacteriaceae family (n = 49). The limit of detection of the ELISA test was 0.5 ng/ml of purified VT1, 5 ng/ml when tested with purified VT2 and between 10^5 and 5×10^7 cells/ml evaluated with ten O157 and non-O157 pure *E. coli*. The limit of detection of the method on samples artificially contaminated is estimated between 1 and 10 VTEC CFU/25g. Finally, when naturally contaminated meat and apple juice samples (n = 50) were screened, Transia Plate Verotoxins method offered a good specificity and sensitivity. In conclusion, the Transia Plate Verotoxins kit represents a good alternative to the Vero Test and Latex Agglutination standard methods, allowing effective screening of food samples.

P061 Evaluation and Development of Methods for Recovery of *Escherichia coli* O157:H7 from Artificially Contaminated Chicken Litter

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Reliable methods for detection of *E. coli* O157:H7 in chicken litter are essential to monitor and initiate an on-farm reduction management of the pathogen. This study was designed to compare and modify the existing methods of FDA BAM, USDA and other protocols to develop optimal methods for detection and isolation of *E. coli* O157:H7 from chicken litter. Three selective enrichment broths, modified trypticase soy broth containing 20 mg/liter novobiocin (mTSB+N), modified EC medium with 20 mg/liter novobiocin (mEC+N) and enterohaemorrhagic *E. coli* enrichment broth (EEB), and 4 selective/differential plating media, sorbitol MacConkey agar (SMAC), SMAC with cefixime and potassium tellurite (CTSMAC), hemorrhagic coli agar (HC) and modified Levin's eosin methylene blue agar (mEMB), were evaluated. The efficacy of immunomagnetic separation (IMS) compared to non-IMS and removal of litter prior to incubation were also studied. Chicken litter samples were artificially contaminated with *E. coli* O157:H7 at low (~10 CFU/25 g) and high (~1000 CFU/25 g) levels and enriched as described above at 37°C for 6 and 16 h. Maximum recovery from samples inoculated at low level and enriched for 16 h was 44%. Removal of litter from sample solutions prior to incubation did not improve recovery of low-level inoculum. The highest recovery from samples with high inoculum and enriched for 6 h was 56%. Prolonged enrichment time, from 6 to 16 h, and the use of IMS increased recovery of *E. coli* O157:H7 from chicken litter. Based on our data, the best method (89% recovery) for recovery of *E. coli* O157:H7 from chicken litter with high level contamination was enrichment in EEB followed by IMS prior to plating on CTSMAC. These data provide some basic information in selecting the appropriate method needed to detect *E. coli* O157:H7 on poultry farms.

P062 Combination of Immunomagnetic Separation and Liposome Immunoassay for the Detection of *Escherichia coli* Serotype O157

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The objective of this study is to develop a novel immunochemical technique for the detection of *Escherichia coli* serotype O157, using immunomagnetic separation (IMS) and liposome immunoassay (LIA) detection. Commercially available immunomagnetic beads with antibodies against *E. coli* O157 covalently bound to the surface of beads were used for selective separation/concentration of the pathogen. Liposomes encapsulating a fluorescent dye, sulforhodamine B, as a detection marker were chemically modified to have anti-*E. coli* O157 antibodies conjugated to their surface. One CFU of *E. coli* O157:H7 spiked into 25 grams of ground beef could be detected after 9 h of enrichment culture followed by 90 min for the IMS-LIA processing. For ground beef spiked with 650 CFU of *E. coli* O157:H7, 6 h of enrichment culture was sufficient to obtain a positive signal by IMS-LIA. The serotypes of *E. coli* O157:NM which produce Shiga toxin 1 or 2 could be detected by IMS-LIA, but other serotypes such as *E. coli* O91:H21 and *E. coli* O32:K:H19, exhibiting different antigenic properties from O157, were not detected. The IMS-LIA technique developed in this work has great potential in assays for *E. coli* serotype O157 in food by improving speed and sensitivity.

P063 Potential for Underestimation of *Escherichia coli* O157:H7 Prevalence in Beef Feedlot Cattle

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The purpose of this study was to evaluate the effect of 24 h storage on *E. coli* O157 recovery from fecal pats collected from a large commercial cattle feedlot. Triplicate samples from 54 fresh fecal pats from the floor of 2 pens were collected. One set of triplicates was processed at TTU within 3 h of collection. One of the remaining sets of samples was shipped overnight to a laboratory in Denver, CO, and processed the next day. The remaining set was held overnight at TTU and processed 24 h after collection. *E. coli* O157:H7 was isolated using pre-enrichment in GN-VCC broth followed by immunomagnetic separation and confirmation. The overall proportion of fecal pats positive on at least one occasion was 46.4%. Only 12.5% of fecal pats were culture-positive as determined by the Colorado laboratory. At TTU, the prevalence was 25% on both sets of samples, indicating pathogen survival after 24 h. However, considerable intra-fecal pat inconsistency was evident. Overall, only 55% of results were consistent across the 3 sets of samples. Of the samples held for 24 h, the same result was reported 71% of the time. Intra-lab (TTU) inconsistency was similar (i.e., 70%). Within fecal pat agreement beyond chance was poor. Kappa statistics (and 95% CL) were 0.1 (0, 3.0) for samples held 24 h, and 0.3 (0, 0.5) for samples processed

within the same laboratory. During previous masked tests using known negatives or positives, we have achieved excellent intra-laboratory agreement. Results indicate that the distribution of *E. coli* O157 in feces is inconsistent and further consideration needs to be given to sampling methods. *E. coli* O157:H7 prevalence estimates based on one-time fecal pat sampling from pens, therefore, are likely underestimates of the actual prevalence.

P064 *Escherichia coli* O157 LPS Contamination in Reusable Sampling Vessels as a Source of False Positive Immunoassay Test Results

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It is a common practice in some food microbiology testing laboratories to use plastic (e.g., polypropylene) containers for sample enrichment and growth. These containers are then typically autoclaved, washed and reused. Media prepared in previously used, washed plastic bottles have tested positive by immunoassay even in the complete absence of any contaminating microorganisms. Because the components of these immunoassays are known not to react with the media, and because there are no organisms present to crossreact with, there must be another explanation for this observation. Studies were conducted to determine if residual bacterial components adhering to the surface of polypropylene containers could serve as a source of false positive immunoassay results. RapidChek®; *E. coli* media was added to new, unused polypropylene bottles, and some of these bottles were inoculated with *E. coli* O157:H7. Both inoculated and uninoculated bottles were incubated. After incubation, the plastic containers containing the media and cultures were autoclaved and the contents discarded. The containers with the inoculated media were washed by one of three methods. The non-inoculated containers were rinsed 3 times with distilled water. Media was then added to each washed plastic container, incubated, and tested with multiple immunoassay test methods. All of the media from plastic containers that previously contained *E. coli* O157 tested positive, regardless of the wash system used. All immunoassay test results from plastic containers that had not been inoculated with *E. coli* were negative. Positive samples were demonstrated to be free from any viable *E. coli* O157 by bacteriological culture methods. Select positive samples were further filtered through a 0.2 μm syringe filter and the resulting filtrates tested positive for the *E. coli* O157 antigen. Additionally, media samples were tested for the presence of bacterial endotoxin by use of the standard Limulus Amebocyte Lysate (LAL) test. Samples testing negative for *E. coli* O157 by use of immunoassay contained endotoxin levels at or below 18.2 units/mL. Immunoassay positive samples reported endotoxin levels above 68,000 units/mL. These data strongly suggest that the reuse of unlined plastic containers may play a role in the occurrence of false positive immunoassay test results. The physical and chemical properties of the heat-stable, lipopolysaccharide O antigen recognized by these immunoassays and their role with respect to testing are discussed.

P065 Comparison of Methods for Detection and Isolation of Cold-stressed *Escherichia coli* O157:H7 in Raw Ground Beef

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A comparison was made of the relative efficiencies of two enrichment media, Rapid Check *E. coli* O157 Enrichment Broth (REB) and modified *E. coli* broth containing novobiocin (mEC+n), and four selective plating media, for detection/isolation of cold-stressed *Escherichia coli* O157:H7 in raw ground beef. Ground beef samples (25 g) were inoculated with 1 to 5 or 10 to 20 CFU of *E. coli* O157:H7 and stored at 4°C for 72 h prior to enrichment. After enrichment at 42°C with aeration or at 35°C with no aeration for 8 or 20 h, the cultures were diluted and directly plated onto BCM O157:H7(+) agar, CT-SMAC, CHROMagar O157, and Rainbow Agar O157 plates. The cultures were also tested with the Rapid Check *E. coli* O157 lateral flow immunoassay cassette and with a multiplex PCR assay amplifying segments of the rfbEO157:H7, fliCh7, stx_1, and stx_2 genes. Generally, after 8 h of enrichment at 42°C in REB and mEC+n, *E. coli* O157:H7 colonies were detected on all four selective agar media, even in samples inoculated at a level of 1 to 5 CFU/25g; however, the number of CFU/ml obtained was ca. 1 to 2 $\log_{10}$ higher using REB compared to mEC+n. *E. coli* O157:H7 was not detected by plating on the selective agars when inoculated at levels of 1 to 5 CFU/g of ground beef after 8 or 20 h of enrichment in mEC+n at 35°C without aeration. In most cases, positive results were obtained with the Rapid Check *E. coli* O157 lateral flow cassette when levels of *E. coli* O157:H7 reached ca. 4 to 5 $\log_{10}$ CFU/ml; however, the organism was detected at levels lower than 4 $\log_{10}$ CFU/ml by the multiplex PCR in both REB and mEC+n enrichments incubated at 42°C for 8 or 20 h. Results of this study indicate that enrichment in REB at 42°C with aeration is superior to enrichment in mEC+n at 35°C without aeration for the detection/isolation of cold-stressed *E. coli* O157:H7 by direct plating, the PCR, and by an immunoassay after 8 h

of enrichment when inoculated at levels of 1 to 5 CFU/25 g of ground beef.

P066 DSC Characterization and Antibacterial Susceptibility of *Staphylococcus aureus* Strains Isolated from Mastitic Milk by 3M Staph Express Count System

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Forty-eight individual cows milk samples obtained from the UWRF dairy farm and a commercial dairy farm in Wisconsin were analyzed for *Staphylococcus aureus* by plating on Baird-Parker Agar (BPA), Mannitol Salts Agar (MSA) and 3M™ Petrifilm™ Staph Express Count Plate (PSE) plates. The presumptive *S. aureus* strains were isolated based on the characteristic colony morphology, gram staining, coagulase and catalase reactions and further characterized using the API Staph identification strips. Six of 48 (12%) samples plated on BPA and 26 of 48 (54%) samples plated on PSE yielded isolates showing characteristic colony morphology, gram reaction, positive catalase and coagulase tests. Six of 12 isolates from BPA and 8 of 34 PSE isolates were coagulase negative. The API Staph profiles confirmed 27 isolates as *S. aureus*, 2 *S. simulans*, 3 *S. hyicus*, and 2 *S. chromogens*. The activity of antimicrobial agents commonly used in mastitis treatment (penicillin, ampicillin, oxacillin, cephalothin, erythromycin, pirlimycin, penicillin + novobiocin, sxt, and tetracycline, as well as vancomycin against the staphylococcial strains) was determined using the Kirby-Bauer disc diffusion method. While the majority of strains 30/34 (88.2%) were sensitive to all the antibiotics tested, 5 (14.7%) strains were resistant to ampicillin, erythromycin, penicillin and pyrlimycin and 3 (8.8%) strains were resistant to ampicillin and penicillin. One strain was resistant to oxacillin. No resistant strains against vancomycin were detected. Four of 14 coagulase negative strains were resistant to ampicillin and penicillin.

P067 Immunoaffinity Columns as a Clean-up Tool for Improving the Detection of Staphylococcal Enterotoxins in Foods

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In food, Staphylococcal Enterotoxins (SEs) are mainly detected by immunoassays, after a water-extraction of the food sample. In order to increase the method sensitivity (down to 0.2 ng/g of food) a dialysis-concentration step of the water extract towards a PEG 30% solution (over-night incubation) has been recommended. This concentration step has also produced false positive ELISA results, decreasing the overall method specificity. Using specific monoclonal antibodies, we have produced immunoaffinity columns for SE purification after the water-extraction step. This new preparation method was applied to artificially contaminated dairy products (mainly raw-milk cheeses spiked with SEA, SEB, SEC, SED or SEE) with a limit of detection by the ELISA test lower than 0.08 ng SE/g of food. Some of those samples were found negative when using dialysis-concentration method. On the other hand, the analysis of non-contaminated dairy and mustard samples, which formerly gave false positive ELISA results with the dialysis-concentration procedure, clearly gave negative ELISA results with the IAC protocol. The interferences were eliminated during the IAC step. Finally, the analysis of one naturally contaminated cheese sample resulted in a clearly positive ELISA signal with the use of the IAC. The use of the IAC has allowed both specificity and sensitivity criteria to be increased in the routine analysis of SEs in foods, reducing the overall analysis time.

P068 Application of Extended Single-reaction Multiplex PCR for Toxin Typing of *Staphylococcus aureus* Isolates in Korea

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Extended single-reaction multiplex PCR (esr-mPCR) was developed in this study to detect staphylococcal enterotoxins (SEs), including SEA, B, C, D, E, H, I, and J. The esr-mPCR requires fewer sets of primers than other conventional multiplex PCRs, in that only two forward primers are used for detecting 8 genes. Therefore, it can be used to detect newly identified staphylococcal enterotoxins (SEs) more readily. The esr-mPCR analysis of 141 isolates of *S. aureus* obtained from abattoir and livestock product samples revealed that 27 of the *S. aureus* isolates were toxigenic, including 2 multitoxigenic isolates. The most prevalent SE type was SEI, followed by A and H. In addition, we investigated the clonal relatedness of toxigenic *S. aureus* isolates by arbitrarily primed multiplex PCR (AP-PCR). AP-PCR analysis of toxigenic *S. aureus* isolates revealed that the discriminatory power of AP-PCR was 9 (D=0.81), 8 (D=0.77) and 10 types (D=0.83) with primers AP1, ERIC2, and AP7, respectively. The combination of 3 each AP-PCR result could rearrange toxigenic *S. aureus* isolates into 10 types and 5 subtypes, with the

D-value of 0.92. Interestingly, our data showed that toxigenic *S. aureus* isolates from different sources had different fingerprinting patterns although some of them carried the same types of SE genes. These data suggest that combinations of esr-mPCR and AP-PCR can provide a more powerful approach for epidemiological investigation of toxigenic *S. aureus*.

P069 Validation of the USDA-ARS Package Rinse Method for Recovery of *Listeria monocytogenes* from Naturally Contaminated, Commercially-prepared Frankfurters

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To validate the utility of the USDA-ARS package rinse method for the recovery of *Listeria monocytogenes*, 100 packages of naturally contaminated, commercially prepared frankfurters were examined using this method and the currently used USDA-FSIS product composite method on the same packages. This lot of frankfurters was previously shown to have contamination with *L. monocytogenes* at a frequency of approximately 16%. The USDA-ARS package rinse method was significantly ($P < 0.01$) more effective than the USDA-FSIS product composite method at recovering *L. monocytogenes* from these packages, with 28 testing positive by the former method and 7 being positive by the latter. Additionally, another 100 packages of frankfurters from the same lot were examined in greater depth, using four methods of sampling and enrichment. First, the fluid exudate was removed and enriched; second, a 25 g composite was removed and processed in accordance with the USDA-FSIS composite enrichment method; third, the USDA-ARS package rinse method was performed on the remaining material; and fourth, the remainder of the frankfurters was enriched. While no method yielded a positive result for every package which was positive by any of the four methods, the USDA-ARS package rinse method was better ($P < 0.05$) than enriching the fluid exudate and the standard USDA-FSIS composite enrichment method at detecting *L. monocytogenes*. These studies demonstrate the superiority of the USDA-ARS package rinse method and make a compelling case for its adoption for routine screening of ready-to-eat products.

P070 Detection of *Listeria* sp. in Meat and Meat Products Using Tecra® *Listeria* VIA™ and Biocontrol VIP® for *Listeria* Immunoassays and a Cultural Procedure

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Outbreaks of human listeriosis have been a cause for concern to the food industry and health professionals. The routine methods for detecting *Listeria* sp. in foods are time-consuming and involve the use of selective enrichment media and culturing on selective agars. In this study, the presence of *Listeria* sp. in 120 meat and meat products samples was investigated by two rapid immunoassays (Tecra® *Listeria* VIA and BioControl VIP® for *Listeria*) and a cultural procedure. Detection of *Listeria* sp. by the cultural method was done according to Pagotto et al. (2001) and detection using rapid methods was done following the manufacturers instructions. The agreement between cultural and rapid methods was stablished at a confidence limit of 95%, according to Norman and Streiner (1994). Seventy-nine samples (65.8%) were *Listeria* sp. positive by at least one of the three methods. *L. monocytogenes* was present in 48.3% of the samples. Sixty-four samples (53.3%) were positive by the cultural procedure. For the rapid methods, 62 (51.7%) were VIA positive and 65 (54.2%) were VIP® positive. Fifty-five samples (45.8%) were positive by the cultural method and VIA simultaneously and 55 (45.8%) by the cultural method and VIP®. VIA detected 5 positive samples that weren't detected by any of the other methods, while VIP detected 7 positive samples. Two samples were detected only by VIA and VIP. There was no statistically significant difference between the cultural procedure and any of the rapid immunoassays. The agreement rate between VIA and the cultural method was 87%, and between VIP and the cultural method was 84%.

P071 Multi-laboratory Comparative Study of a PCR-based System and the Standard Cultural Methods for the Detection of *Listeria monocytogenes* in Foods

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The BAX® system is an automated screening method that uses Polymerase Chain Reaction (PCR) technology to detect *L. monocytogenes* in foods. Traditional screening methods are based on bac-

terial behavior, such as phenotypic or antibody response, which can present problems because of cross-reactivity of related organisms. The BAX® system, however, focuses on the genetic makeup of the bacteria. It will detect a DNA fragment that is unique to the target organism. Even at very low concentrations, these fragments can be amplified by the PCR to levels detectable by the BAX® system cycler/detector. A multi-laboratory study was conducted to compare the automated BAX® system to the standard cultural methods for the detection of *Listeria monocytogenes* in foods. Six food types — frankfurters, brie cheese, smoked salmon, ground beef, radishes, frozen peas — at 3 inoculation levels were analyzed by each method. A Chi-square analysis of each of the 6 food types, at the 3 inoculation levels tested, was performed. For all foods, with the exception of radishes, the BAX® system performed as well as or better than the standard reference methods. Additionally, the combined sensitivity for all foods, with the exception of radishes, was 94% for the test method and 93% for the standard methods. Therefore, this PCR-based system is an acceptable alternative to the current standard methods.

P072 Evaluation of a Harmonized Enrichment Method for the Detection of *Listeria monocytogenes* by Two Individual Assays

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The purpose of this study was to compare the bioMérieux VIDAS *Listeria monocytogenes* 2nd generation (LMO2) and *Listeria* (LIS) immunoassays to the FDA-BAM, AOAC or USDA/FSIS reference methods for their respective foods, with use of a harmonized, two-step enrichment. The study consisted of inoculated product testing of 600 individual samples in 15 foods over a wide variety of food types including dairy, vegetable, seafood, and both raw and processed meat products. Each food type was tested using both immunoassays and the appropriate reference method. All presumptive positive samples were confirmed via the reference method. Overall, the LMO2 and LIS assays demonstrated 88% sensitivity and the reference methods demonstrated 87% and 86% sensitivity, respectively. Based on these data, both assays demonstrate statistical equivalence for detecting *Listeria monocytogenes* following a harmonized, two-step enrichment method.

P073 Comparison of a Chromogenic Agar to Conventional Agar Media for the Detection of *Listeria monocytogenes*

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Listeria monocytogenes is a Gram-positive, rod-shaped bacterium which has been implicated in a number of cases of foodborne illness. In October 2002, 27.5 million pounds of ready-to-eat turkey and chicken products contaminated with *L. monocytogenes* were recalled in the United States. Unfortunately, the standard methods for isolation do not allow for rapid and direct identification of *L. monocytogenes*. The present study compares the use of a chromogenic agar, RAPID' L.MONO® (RLM) to standard methods agars. RLM is a selective medium that inhibits most other Gram-positive organisms as well as inhibiting Gram-negative organisms, yeasts and molds. Colonies of *L. monocytogenes* are grayish to dark blue with no yellow halo, while colonies of *L. ivanovii* are greenish-blue with a yellow halo. Other *Listeria* species produce white colonies. In this study, four foods (brie cheese, surimi, tossed salad, and deli turkey) were spiked with *L. monocytogenes* and tested by the standard methods, including streaking a RLM plate from the primary enrichment. On the standard method agar plates, typical *Listeria* morphology was observed. Similarly, the chromogenic agar plates produced approximately the same number of *Listeria* colonies, of which *L. monocytogenes* were distinguished by the grayish to dark blue color. Each of these colonies was subsequently confirmed as *L. monocytogenes*. The color differentiation of the *L. monocytogenes* on the RLM plate allows for confirmed results in 48-72 h, as opposed to the 72-96 h needed in the standard methods.

P074 A Method for Evaluating Changes to UVM Media for Improving the Growth of *Listeria monocytogenes*

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A microplate-based method was developed for evaluating the efficacy of additives for improved growth of *L. monocytogenes* in UVM. Essential compounds for *L. monocytogenes* growth or compounds that have demonstrated ability to improve bacterial growth under stressful conditions were tested by two-fold serially diluting the compounds and distributing them across the length of a microplate inoculated with *L. monocytogenes*. Multiple replicates were run with two strains of *L. monocytogenes* and were read at 595 nm using a microtiter plate reader. The repeated measures data for each concentration of the compounds tested were averaged down each column of the microplate and analyzed using SAS Institute software. The osmoprotectants glycine betaine and carnitine did not improve the growth of *L. monocytogenes* in UVM. Also, the iron containing compound, hemin, was ineffective at improving the growth of *L. monocytogenes* in UVM. Alpha-ketoglutarate (alpha-KG) at concentrations of 0.1 to 3.1 g/L was found to improve the growth of

L. monocytogenes in UVM. Above 3.1 g/L, alpha-KG was found to be inhibitory to growth. Sodium pyruvate at concentrations of 0.1 to 6.3 g/L was found to improve the growth of *L. monocytogenes.* Pyruvate was inhibitory to growth at 12.5 g/L and greater concentrations. Growth was also improved with the addition of a minor elements solution providing a final concentration of 400 μg/L $FeSO_4 \times 7H_2O$, 15 μg/L $MnSO_4 \times 2H_2O$, and 15 μg/L $NaMoO_4 \times 2H_2O$. These media changes accelerate the growth and improve the recovery of *L. monocytogenes* from UVM enrichments.

P075 Evaluation of the MicroFoss System for the Detection of *Listeria* Species in Environmental Samples

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The MicroFoss system was evaluated for its ability to detect *Listeria* species in environmental sponge samples. The sensitivity and specificity of the MicroFoss were determined in relation to a standard culture method for *Listeria* detection. The sensitivities of both the MicroFoss and standard culture methods were identical at 90.3%. The MicroFoss system detected *Listeria* species in ten samples which were not detected by culture, and vice versa. This was likely due to uneven distribution of low levels of *Listeria* organisms in the split sponge samples used to assess the performance of these test methods. The specificity value determined for the MicroFoss system was 75.3%. The majority of microbes causing false positive results in the MicroFoss system were *Bacillus* species, which were readily distinguishable from *Listeria* species by a simple Gram stain and morphological features. *L. monocytogenes* (MicroFoss – 89.2%, Culture – 87.1%) and *L. innocua* (MicroFoss – 9.7%, Culture – 9.6%) were the most common isolates of *Listeria* detected by the two test methods. *L. monocytogenes* and *L. innocua* were the two predominant *Listeria* species detected by both methods in the environmental samples, with *L. monocytogenes* being the most commonly detected isolate. The highly comparable results and rapid nature of the MicroFoss system demonstrate its effectiveness as a detection system for species of *Listeria* in environmental samples. The fact that the sensitivity of the MicroFoss system was identical to that of the culture method and that the *Listeria* results were obtained within 48 h of testing support the use of the MicroFoss as an alternative rapid method for screening large numbers of environmental samples for *Listeria* spp.

P076 Development and Optimization of a Real-time PCR Assay for the Detection of *Listeria monocytogenes* Using the LightCycler® System

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In the LightCycler® (LC) System, rapid-cycle PCR in conjunction with fluorimeter-based closed-tube PCR assays have provided a rapid and sensitive method for real-time identification and quantification of PCR products. The 16S/23S ribosomal RNA intergenic spacer region has been successfully used as a target for the development of species-specific PCR/DNA probe assays for a range of microbial pathogens. In this study, a species-specific assay for *Listeria monocytogenes* targeting the 16S/23S rRNA intergenic spacer region was developed for use in the Lightcycler®. FRET (Fluorescence Resonance Energy Transfer) adjacent hybridization probes specific for the identification of *L. monocytogenes* were designed for use in the Lightcycler®. These probes consist of two oligonucleotides that hybridize adjacent to the target DNA sequence, one of the oligonucleotides is 3'-labeled with fluorescein while the other is 5'-labeled with the lightcycler dye LC-Red 705. Application of the LC assay to DNA extracted from panels of food and dairy bacteria and *Listeria* species has shown the assay to be specific for *L. monocytogenes* target DNA. Optimization of the assay parameters resulted in the ability to reliably detect 100 fg levels (~ 30 cells) of *L. monocytogenes* DNA. The fluorescence technology used in this assay has potential to allow the development of a high-throughput molecular diagnostic test for *L. monocytogenes* in both the clinical and food processing environment.

P077 Multiplex PCR for Serotype Identification of *Listeria monocytogenes*

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Of 13 known serotypes of *Listeria monocytogenes,* three (1/2a, 1/2b, and 4b) are most frequently associated with human infections. Serotyping of *Listeria monocytogenes* is useful for first level discrimination, before more sensitive subtyping methods are applied for surveillance and outbreak investigations. However, reagents for serotyping *Listeria* are available only in a few specialized public health laboratories in the world. We investigated the possibility of using a DNA-

based method. Only 4 flagellar antigens have been described for *Listeria* spp. The flaA genes encoding the four antigenic types were sequenced and compared. Using this information, we designed a four-primer multiplex PCR reaction for flagellar antigen typing of *L. monocytogenes*. Another primer pair, which amplified an 853-bp fragment of gltA, a surface antigen gene specific to *Listeria* serotypes 4b, 4d and 4e, was incorporated into the multiplex PCR scheme to allow the differentiation of serotype 4b from 1/2b. After optimization, the multiplex PCR reaction was evaluated against a panel of 60 *L. monocytogenes* strains representing various serotypes. All the tested *L. monocytogenes* strains, except for one that showed a minor deviation in the size of its four amplicons, generated the PCR pattern expected for the corresponding serotype. The Multiplex PCR approach should be useful for serotype identification of *L. monocytogenes*, particularly in those laboratories that do not have access to serotyping reagents.

P078 DSC Comparison of MPN Procedures Designed for Recovery of Low-level Healthy and Injured *Listeria monocytogenes* in Ready-to-Eat Foods

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Recent outbreaks of *Listeria monocytogenes* have been attributed to low levels of contamination in food products. Existing methods for the detection of *L. monocytogenes* lack the sensitivity needed for detection of low-level healthy or injured cells. In this study the current USDA/FSIS MPN procedure utilizing UVM broth was compared to a modified system using a) *Listeria* Repair Broth (LRB) and b) UVM combined with LRB with a 4 h recovery period prior to the addition of selective agents (UVM/LRBS). Three ready-to-eat (RTE) foods were analyzed; hot dogs, smoked salmon and fermented sausage. The products were inoculated with ≤ 10 *L. monocytogenes* cells/g. The methods used followed the USDA/FSIS MPN procedure. Isolation and identification of *L. monocytogenes* was performed by plating MPN enrichment to Oxford and Palcam agar plates and appropriate biochemical testing. The MPN system using LRBS detected *Listeria* at a level of approx 10 MPN/g significantly outperformed procedures using UVM and UVM/LRBS when the food was inoculated with <10 CFU/ml of healthy cells. Higher levels of detection were obtained from fermented sausage than from the hot dogs or smoked salmon. It appears that LRBS is able to detect low-levels of healthy *Listeria* better than UVM or UVM/LRBS. Detection of low-levels of injured *Listeria* was improved by use of UVM/LRBS when compared to UVM or LRBS, detecting approx. 15 MPN/g when the food was inoculated with < 100 CFU/ml of *Listeria monocytogenes* with an injury level of 99.6%. Continued research must focus on improving the sensitivity of detection of *Listeria* in high risk foods.

P079 Use of Sequence Typing for Characterization of Virulence Factors and for the Development of a Novel Molecular Typing Scheme for *Listeria monocytogenes*

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The objectives were to develop a multi-locus sequence typing (MLST) scheme for *Listeria monocytogenes*. Comparison of sequence variation between the housekeeping (for which allelic variations are typically selectively neutral) and virulence genes (for which allelic variations often represent selective advantage) will enable assessment of virulence genes for critical hypervariable loci, for horizontal gene movement, and for particular alleles that identify genotypes of increased virulence. In the MLST method, the internal fragments (approximately 450 to 500 bp) of 7 housekeeping genes or less are amplified and sequenced to determine the allelic profile of each isolate. For each housekeeping gene, the different sequences found between all the isolates are each assigned a distinct allele. Each isolate is therefore unambiguously characterized by the combination of alleles for the 7 housekeeping genes loci. A set of 200 isolates representing diversity in origin, date of isolation and source (clinical, environment, food) have been chosen. A subset (n=29) has been selected to ensure the feasibility of the MLST approach with respect to discrimination potential. Twenty sequence types corresponding to the 7 alleles chosen were obtained for the subset isolates. Four recurring sequence types were identified. MLST data are being compared to PFGE and ribotyping data. MLST has the potential of becoming the method of choice for population genetics of bacterial species. Rapid, unambiguous electronic transmission of sequence data and precise, quantitative analyses of large databases will be available with the MSLT approach.

P080 Twenty-four Hour Enrichment and Detection of Stressed *Listeria monocytogenes* on Stainless Steel Surfaces Using PATHIGEN® *Listeria* Broth and the PATHIGEN *Listeria* Test

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One important aspect of *Listeria* prevention is an effective monitoring program for the presence of *Listeria* on environmental surfaces. In this study,

we evaluated the ability of four different selective enrichment media to recover and enrich dilutions of stressed *L. monocytogenes* cultures. The media tested included modified *Listeria* Enrichment Broth (mLEB), Fraser Broth, BCM® *Listeria monocytogenes* Pre-enrichment Broth (BCM), and PATHIGEN *Listeria* Broth (PLB). The cultures were stressed by application to a stainless steel surface followed by drying at room temperature for up to 4 h. Following sampling of the stainless steel surfaces with a pre-wetted swab, individual swabs were enriched in each media at 30 or 37°C for 24, 36, and 48 h. The enrichment broths were then tested for the presence of *L. monocytogenes* using the PATHIGEN *Listeria* Test. All PATHIGEN *Listeria* Test positive results were confirmed by plating and biochemical analysis. The results of this testing showed that recovery and detection of stressed *L. monocytogenes* from the stainless steel surface within a 24 h timeframe could only be accomplished with use of PLB. Recovery and detection of *L. monocytogenes* with mLEB or Fraser broth could be achieved only with cells collected immediately after drying. BCM recovered cells when dried on a surface for 4 h, but only following a 48 h enrichment. This data demonstrates the ability of the PATHIGEN *Listeria* Test, combined with PATHIGEN *Listeria* Broth, to detect environmental *Listeria* contamination by use of a 24 h enrichment, in contrast to traditional enrichment and plating methods that can take up to 4 days to yield an equivalent result.

P081 Comparative Analysis of a Rapid Immunoassay to the Standard Cultural Methods for the Detection of *Listeria monocytogenes* in Ready-to-Eat Foods and Dairy Products

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Contamination of ready-to-eat (RTE) foods and dairy products with *Listeria monocytogenes* continues to be a significant problem in the food industry. This study compared a rapid immunoassay, the bioMérieux VIDAS LMO2, to the current standard methods for a variety of RTE foods and dairy products. For the RTE products, 225 total samples of surimi, cauliflower, beef frankfurters, chicken frankfurters and roast beef were analyzed by the immunoassay and the FDA Bacteriological Analytical Method. The results showed 91% sensitivity for both the LMO2 assay and the standard method. For the dairy products, 180 total samples of milk, Velveeta cheese, brie cheese and ice cream were analyzed by the test kit and the AOAC Official Method for the detection of *L. monocytogenes*. The results showed 93% sensitivity for the LMO2 assay and 90% sensitivity for the AOAC method. These data suggest that the LMO2 assay is comparable to the current standard methods for detecting *L. monocytogenes* in RTE foods and dairy products.

P082 Evaluation of a Lateral Flow Immunoassay for the Detection of *Salmonella* in Raw Beef

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Salmonella is an important human pathogen that has been implicated as a major cause of foodborne illness worldwide. Several rapid methods have been developed for detection of *Salmonella* species. In this study, we examined the performance of the RapidChek *Salmonella* lateral flow immunoassay with the RapidChek 24 and 48 h enrichment procedures to detect *Salmonella* spp. in raw ground beef. Ground beef samples were spiked with 3 *Salmonella* species at 0, 0.24, 0.8, and 2.4 CFU/25g sample in replicates of 5. The samples were cold stressed for 48 h at 4°C, and subsequently enriched using the FSIS/MLG protocol recommended for raw beef, or the RapidChek 24 h protocol using the RapidChek *Salmonella* Enrichment Broth. Following the respective enrichments, samples were screened using the RapidChek device. Samples showing a positive immunoassay result were streaked to selective media and further confirmed by latex agglutination. In addition, all samples from the FSIS/MLG enrichment procedure were streaked to selective media and latex confirmed as the primary detection method. The FSIS/MLG method and the RapidChek 48 h method reported 30 of the 45 spiked samples positive for *Salmonella*. The RapidChek 24 h method reported 37 of the 45 spiked samples as positive. All immunoassay positive samples were confirmed positive. The results of this study suggest that the RapidChek 24 and 48 h methods demonstrate similar performance to the FSIS/MLG 48 h method for the detection of *Salmonella* in raw beef. The RapidChek 24 h method provides an improvement in time and labor for *Salmonella* testing.

P083 Use of Automated Immunomagnetic Separation for Detection of *Salmonella* in Cattle Feces

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Cattle feces were tested for the presence of *Salmonella* by use of Automated Immunomagnetic Separation (AIMS) that utilized the Dynal Bead Retriever™ and Dynabeads anti-*Salmonella*. AIMS was compared with a standard protocol (SP) which consisted of enrichment in Buffered Peptone Water

for 16–24 h at 37°C, followed by enrichment in 2 selective broths for 18–24 h (Rappaport Vassiliadis broth at 42°C and Mannitol Selenite Cystine broth at 37°C). Selective enrichments were plated onto Xylose Lysine Desoxycholate Agar and Bismuth Sulfite Agar. A total of 60 fecal samples were examined using both methods. *Salmonella* was detected by both AIMS and the SP method in 25 fecal samples, with AIMS detecting *Salmonella* in an additional 3 fecal samples that were negative by the SP, and 4 fecal samples were found to contain *Salmonella* by use of the SP, but were negative using AIMS. The AIMS procedure provided results 24 h prior to the SP and *Salmonella* colonies were easier to detect on selective plating media after the AIMS procedure. AIMS was useful for the detection of *Salmonella* in cattle feces, with results obtained faster than when the SP was used.

P084 Comparison of Electrochemical, Impedance and Optical Sensors for Rapid Detection of Live *Salmonella* Typhimurium in Food Products

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Three sensing technologies, electrochemical, impedance and optical, were compared in development of a rapid method for detection of live pathogenic bacteria in foods. *Salmonella* Typhimurium in chicken carcass wash water or milk was directly incubated at 37°C for 15 h in a microincubator filled with either selective (selenite cystine/mannitol broth) or non-selective (brain heart infusion broth) growth medium. The mixture of growth medium and samples with *S.* Typhimurium was monitored for its change in electrical current, impedance and absorbance by use of electrochemical cyclic voltammetric scan, three-electrode frequency spectral scan, and a fiber optic oxygen sensor, respectively. A detection time was defined in the curves of peak current, impedance and absorbance vs time when a sharp decrease occurred, which was inversely correlated to the initial cell number of *S.* Typhimurium in the sample. The results showed that all three sensing technologies effectively detected the electrochemical, impedance and optical features of the bacterial growth. Linear regression equations were found between the detection time and the logarithmic values of the initial cell number, with $R^2 \geq 0.98$. When the cell number was from 1 to 10^6 CFU/ml, the detection time was from 15 down to 3 h. All three methods could detect as low as 1 CFU/ml without any pretreatment of samples. Food samples can be directly placed into the microincubator and live pathogenic bacteria can be enumerated in <15 h. The fiber optical sensor was simple and cost-effective, the electrochemical sensor was more accurate, and the impedance sensor provided more data for microbial growth curve.

P085 A Rapid 24-hour Enrichment Protocol for *Salmonella* in Foods

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Rapid enrichment and detection of *Salmonella* in foods has been one of the primary interests in the food industry for the safety of consumers. Most rapid detection methods use conventional 3-step 2-day enrichment protocols as recommended by USDA/FSIS or FDA/BAM, which require at least 48 h from initiation of analysis to presumptive test results. The present studies were conducted to evaluate the efficiency of a rapid 24-h enrichment protocol for *Salmonella* in foods, which uses a proprietary RapidChek *Salmonella* Enrichment Broth, compared to conventional enrichment methods. Cells of *S.* Typhimurium, *S.* Maarssen and *S.* Montevideo were spiked into raw ground beef at four levels, from 0–10 CFU per 25 g sample, in replicates of 10 (n=200). After enrichment, the samples were streaked to BGS and XLT4 plates, and presumptive positives were further confirmed by *Salmonella* latex and other biochemical tests. From the spiked samples, the 24-h method recovered 65 confirmed positives out of 90 samples, while the 48-h method recovered 50 positives out of 90 samples. In a separate experiment, various food matrices, including Deli turkey, cabbage, cantaloupe, raw salmon, raw boneless beef and turkey, raw ground pork, chicken and beef, were screened for natural contamination of *Salmonella* by use of the two enrichment protocols in replicates of 5 to 10 for each matrix. Out of 65 samples tested for each method, both methods detected 2 positive *Salmonella* samples. These studies indicate that the rapid 24-h enrichment protocol is comparable or more efficient in recovering *Salmonella* from foods, while providing a significant time and labor savings.

P086 Evaluation of Methods for Recovery of *Salmonella* from Poultry and Swine Feed

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Numerous studies have been conducted to compare and improve microbiological techniques for detection and isolation of *Salmonella* from foods for human consumption, but few have focused on animal feeds. The objective of this study was to

determine the most effective technique for the detection and isolation of *Salmonella* from poultry and swine feed. Procedures for recovery involved pre-enrichment in lactose broth (LB) and direct enrichment in Rappaport-Vassiliadis (RV), selenite cystine (SC), and tetrathionate at 35°C (TT35) and at 42°C (TT42) in conjunction with differential plating on brilliant green agar (BG), bismuth sulfate agar (BS), Hektoen enteric agar (HE) and xylose-lysine-tergitol 4 agar base (XLT). The feed samples were artificially contaminated with *Salmonella* spp. at low levels (~10 CFU/25g of sample). The most favorable method for recovery (90%) of *Salmonella* from swine feed without antibiotic involved direct enrichment in SC followed by isolation on BS or direct enrichment in TT35 streaked on BG. High recovery (90%) was also obtained when samples were pre-enriched in LB followed by enrichment in TT35 or RV and streaked on BS or HE for isolation. The recovery (90%) in swine feed with antibiotic required direct enrichment in RV or TT35 and plating on BS. The recovery was equally effective when pre-enriched in LB followed by enrichment in TT35 or TT42 and streaking on HE or BS. In examining poultry feed the best methods (100% recovery) were direct enrichment in SC or TT35 with isolation on BS, HE, or XLT, with similar recovery (100%) when pre-enriched in LB followed by TT35 with isolation on BS. These data show that selection of appropriate protocols to isolate *Salmonella* from animal feeds is strongly dependent upon the type of feed.

P087 A Rapid Test Method for the Detection of *Salmonella* in Dairy Factory Environmental Samples

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Salmonella spp. are a well known pathogen group associated with many food poisoning outbreaks. Surveillance of the dairy factory site environment for the presence of *Salmonella* is used to monitor and take action if necessary to minimize risk of contamination from the environment. Rapid and sensitive methods to survey the environment are essential to ensure a speedy response to preserve the integrity of the dairy site and its products. Such methods allow the testing laboratory to test more samples and free personnel for other tasks. In this study, 191 environmental swabs were taken from dairy factories to determine the presence of *Salmonella* spp. The swabs were enriched in Modified Buffered Peptone Water (MBPW). Portions of the enrichment were then examined for the presence of *Salmonella* spp. by two methods, one using the TECRA ULTIMA™ *Salmonella* test kit and the other using the FDA/BAM method (Other Foods Chapter 5, FDA/BAM 8th Edition). Results showed the TECRA ULTIMA™ rapid method to be comparable to the FDA/BAM cultural method for recovery of *Salmonella* spp., with 99.48% agreement. No false positives were observed. Three samples returned positive results compared to four for the cultural method. The undetected positive was at the lower limit of sensitivity (1cell/25g) for both methods. The ULTIMA™ method requires only a single selective enrichment step and provides presumptive results within 36 h compared with over 60 h for the standard cultural method. This allows much better use of laboratory time to address critical issues such as the control of environmental reservoirs of *Salmonella*.

P088 Real Time PCR Analysis of Primary *Salmonella* Enrichments from Broiler Carcasses Using the R.A.P.I.D. IT – *Salmonella* Assay

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To determine the efficacy of a real time PCR *Salmonella* assay (R.A.P.I.D. IT-*Salmonella*, Idaho Technology, Inc.) a set of comparison experiments with another PCR-based assay as well as standard cultural methodology was performed. Broiler chicken carcass rinse samples from a variety of unspecified U.S. sources were analyzed for salmonellae by use of the FSIS screening PCR method (BAX-*Salmonella*) alongside the R.A.P.I.D. IT-*Salmonella* (Idaho Technology, Inc.) and FSIS culture methodology. A total of 111 samples taken during the summer over a 6-week sampling period were shipped overnight, refrigerated while shipped, and analyzed. Results were as follows: R.A.P.I.D. IT-*Salmonella* positive = 19/111; BAX *Salmonella* 17/111, FSIS Culture 19/111. All positive samples matched between the R.A.P.I.D. IT-*Salmonella* assay and the FSIS culture method. The 17 samples found positive by the BAX *Salmonella* assay were also found positive by the R.A.P.I.D. IT-*Salmonella* and the culture method. Two discordant samples, taken during two different weeks, were all culture and serology confirmed. The R.A.P.I.D. IT-*Salmonella* assay includes a short, manual DNA extraction protocol from enrichment cultures, followed by a PCR amplification and analysis step of approximately 30 min per sample. Currently, determination of positive and negative threshold values using the R.A.P.I.D. is accomplished by fluorescence output graph examination. While not a high sample throughput format, (e.g., 96 well plate), the R.A.P.I.D. IT-*Salmonella* assay offers an efficacious means to screen small numbers of samples of broiler carcass Salmonellae enrichments that is time efficient and does not require extensive heating or cooling steps for cell lysis and DNA extraction.

P089 Detection of *Salmonella* from Chicken Rinse and Chicken Franks with Electrochemiluminescence and Automated PCR Assays

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Processed chicken carcass rinses were sampled for *Salmonella* after buffered peptone (BP) rinse or after the addition of either 3 or 0.3 *Salmonella*/mL of rinse. Chicken franks were cut into 10 g portions and stomached with 90 mL of BP and sampled for *Salmonella* directly or after the addition of either 31 or 3 *Salmonella*/mL. The entire experiment was replicated twice. All samples were assayed for *Salmonella* by the cultural procedures of FSIS, by the IGEN PATHIGEN® *Salmonella* electrochemiluminescence procedure after 18 to 20 h in BP and after an additional 24 h in RV, and by the Qualicon BAX® automated PCR *Salmonella* assay after 18 to 20 h in BP. Briefly, the The PATHIGEN *Salmonella* Test uses a sandwich immunoassay format in which a polyclonal antibody specific for *Salmonella* binds the organism to a paramagnetic microparticle and a second polyclonal antibody specific for *Salmonella* is labeled with a compound which is excited at the surface of an electrode and emits light when *Salmonella* is present. The PATHIGEN® takes about 2 h to run. The automated BAX® assay procedure is as follows: overnight growth (16 to 18 h) of sample in buffered peptone broth at 35°C, transfer of sample to lysis tubes, incubation and lysis of cells, and transfer of sample to PCR tubes, which are placed in a cycler/detector which runs automatically. The BAX® PCR/detection assay takes about 4 h. From chicken rinse samples, *Salmonella* were recovered from all (14) high inoculum samples by all methods and from all but one of the low inoculum samples with the PATHIGEN from BP. From chicken franks, *Salmonella* were recovered from all (14) high inoculum samples by all methods and from 13 of 14 samples by the BAX and PATHIGEN as compared to 14 of 14 cultural samples. Both the PATHIGEN and BAX assays were easy to run and were highly effective alternative methods to the more time consuming standard cultural procedure.

P090 Evaluation of a Classic PCR Method and an Electrochemical ELISA Method Coupled with an FIA System for the Detection of *Salmonella* in Meat

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Salmonella is currently considered the most common cause of foodborne infection in industrialized countries, where contamination of meat products is especially common. According to European legislation, *Salmonella* must be absent in 25g of product. The standard method (ISO/DIS 6579, 2001) involves sequential cultural steps that are time-consuming, taking more than 4 to 5 days for *Salmonella* detection. Rapid methods for the realization of prevention programs are needed. The aim of this study was the evaluation, by comparing with the ISO method, of two different techniques to detect salmonellae in meat: an electrochemical ELISA (sandwich format) using electrochemical detection coupled with a FIA system and a PCR method. The latter, carried out with ST11 and ST15 primers according to Aabo (1993), was performed for the research of specific genomic fragment (429 bp) using an internal control (240 bp) performed by the ST15 primer and the IS-ST11 recombinant primer according to Rijpens (1999). The experiments were carried out using experimentally and naturally contaminated samples of meat. Both methods showed good correlation with the ISO method, good selectivity for salmonellae, no cross-reactivity with other bacteria and were sensitive and able to reduce drastically the analysis time. it was possible to detect 1 to 10 salmonellae in 25 g of product after only 5 h of pre-enrichment step. It can be concluded that both tests are quick, efficient, and easy to carry out; permitting the simultaneous analysis of numerous samples, they constitute a valid assay to perform both monitoring programs and controls for the application of a HACCP program in the food production industry.

P091 Duplex SYBR-Green Real Time PCR for Detection of *Salmonella* spp. and *S.* Enteritidis in Poultry

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Since the standard cultural method (ISO 6579) requires up to five days for detecting *Salmonella* in food, different methods have been developed to reduce the time. Many of the PCR assays employ either visual scoring of ethidium bromide-stained agarose gels or post-PCR hybridization-capture methods that are labor intensive, time consuming and difficult to automate. Recently, the use of double stranded DNA binding dye SYBR Green I for the automated detection of PCR product allowed an early and simple approach to the PCR. The aim of this work was to develop a simple duplex SYBR-Green Real-time PCR for simultaneous detection of *Salmonella* spp. (by Styinva-JHO primers), and *S.* Enteritidis (by Sefa primers), using the detection of Tm (melting temperature) as the discriminating factor. The experiments were conducted with

samples of chicken experimentally contaminated with *S.* Enteritidis and commercially available poultry samples. The Tm of the non-*Salmonella* Enteritidis amplicons in the presence of the four primers showed only a peak corresponding to the Styinva-JHO primers amplicon (Tm = 77.03 ± 0.09); in contrast, the Tm of the *S.* Enteritidis amplicon showed a large peak corresponding to SefA primers amplicon (Tm = 83.29 ± 0.089) and another small peak corresponding to Styinva-JHO amplicon. The results demonstrate that the automated duplex SYBR-Green Real-time PCR, when integrated with the study of the melting curve, is efficient, sensitive, and rapid in detecting *Salmonella* spp. and presumptive *S.* Enteritidis without any cross-reactivity for other bacteria.

P092 Evaluation of Methods for Recovery of *Salmonella* spp. from Dairy Environmental Samples

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Current official methods for detection and isolation of *Salmonella* spp. are mostly designed for use with foods. The objective of this study was to identify the most favorable methods for detection and isolation of *Salmonella* spp. from dairy environmental samples. Pre-enrichment in lactose broth (LB) versus direct enrichment (no pre-enrichment) prior to selective enrichment in Rappaport-Vassiliadis (RV), selenite cystine (SC), and tetrathionate broth incubated at 35 (TT35) and 42°C (TT42) in conjunction with differential/selective plating on brilliant green (BG), bismuth sulfite (BS), Hektoen enteric (HE) and xylose-lysine-tergitol 4 agar base (XLT) were evaluated for their ability to recover *Salmonella* spp. from artificially contaminated sample. Bedding, soil, manure, water and flies were artificially contaminated with *Salmonella* spp. at 1 to 2 CFU/g of sample. Pre-enrichment in LB did not improve recovery of *Salmonella* spp. from samples except from trough water and it decreased recovery in several sample types. Direct enrichment in RV followed by isolation on HE or XLT recovered *Salmonella* spp. from bedding in 100% of samples. The best method to recover *Salmonella* spp. from soil (100% recovery) was direct enrichment in TT42 followed by isolation on BS, HE or XLT. Direct enrichment in SC or TT42 followed by streaking on XLT (100% recovery) was as effective as pre-enrichment in LB followed by enrichment in RV or TT42 with plating on BS for isolation of *Salmonella* spp. from cow manure. The highest recovery of *Salmonella* spp. from water without pre-enrichment in LB was 30%. The best methods (100% recovery) to detect and isolate *Salmonella* spp. from water were pre-enrichment in LB followed by enrichment in RV or TT42 with isolation on BG, BS, HE or XLT or by enrichment in TT35 with isolation on XLT. The most effective method (80% recovery) to recover *Salmonella* spp. from fly samples was direct enrichment in RV followed by streaking on BS plates. The most effective media combinations for isolation of *Salmonella* spp. from dairy environmental samples depended upon the type of samples. Generalization of protocols for recovery of *Salmonella* spp. from dairy farm samples may result in poor recovery, increased recovery time and increased sample processing costs due to unnecessary isolation steps.

P093 DSC Validation Assay of Two Immunodiagnostic Methods (VIDAS SLM and VIDAS ICS) and Two Classical Methods (SP-VG-M002 and NMKL 71) for *Salmonella* Detection in Fecal Samples from Porcine Origin

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The aim of this study was to compare two immunodiagnostic methods and two classical methods of detecting *Salmonella* in naturally contaminated porcine fecal matrices (cecal contents taken at abattoir, feces and overshoes sampled in farms, sludge from porcine farms). The protocols for environmental samples for VIDAS SLM and VIDAS ICS were strictly followed as described by Biomerieux. The two classical methods were: 1. SP-VG-M002, Belgian reference method for detection of *Salmonella* in foods, based on the use of a modified semi-solid Rappaport-Vassiliadis broth and, 2. NMKL 71 method, imposing the use of Rappaport-Vassiliadis enrichment broth. The VIDAS SLM recovered most of the positive results and so was chosen as reference method. Results evaluated three comparisons: SP-VG-M002 versus VIDAS SLM (n=155), VIDAS ICS versus VIDAS SLM (n=83) and NMKL71 versus VIDAS SLM (n=94). Proportions of agreement were 81.3, 68.7 and 80.9%, while relative sensitivity was respectively 41.9, 30.6 and 47.1%. Relative specificities were judged good, with values ranging from 96.4 to 100%. No differences were statistically significant. Concerning the agreement evaluation, Kappa values were evaluated as intermediate but significantly different from zero (0.45 for SP-VG-M002, 0.31 for VIDAS ICS to 0.53 for NMKL 71). In conclusion, for these fecal materials, VIDAS SLM was shown to be more suitable for *Salmonella* detection than the other methods studied. An explanation of this could be the use of Muller-Kauffman as enrichment broth before VIDAS SLM, leading to more positive results than classical methods.

P094 DSC Fluorescent *In Situ* Hybridization for the Culture-independent Detection of *Campylobacter jejuni*

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Campylobacter jejuni is recognized as the leading cause of foodborne disease in North America. The detection of *C. jejuni* using routine culturing methods is difficult because special media (containing blood) and gas environments (microaerophilic) are required. The objective of this work was to apply fluorescent in situ hybridization (FISH) for the culture-independent detection of *C. jejuni* in chicken meat and environmental samples. A rhodamine labelled oligonucleotide probe targeting the 16s rRNA of *C. jejuni* was evaluated for its specificity in the FISH-assay with 20 *C. jejuni* strains and 16 non-*Campylobacter* species. The probe showed a positive signal with all *C. jejuni* while no signal was detected with other microorganisms. Buoyant density centrifugation (BDC) is a simple technique used to separate cells and particles through a gradient medium. Since debris from samples will interfere with the FISH signal, the BDC method was evaluated for its ability to remove such background without any loss of target bacteria. Pure culture suspensions of *C. jejuni* at 10^2, 10^3 and 10^4 CFU/ml were subjected to BDC. Cell numbers determined by plate counting were identical for all samples before and after BDC treatment ($P < 0.001$). In samples with 10^3 CFU/ml, a significant increase in total cell counts was observed ($P = 0.025$). When tested with chicken homogenate, BDC was effective in reducing interfering large sample debris. The data from this project provide basic information required for the further development of a culture-independent detection method that can be used for poultry and environmental samples.

P095 *In Vitro* Invasive Assay for *Campylobacter jejuni* from Raw Broiler Carcass Rinses

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We have developed an in vitro invasion assay model for *Campylobacter jejuni*, using a β-lymphocyte derived mouse hybridoma cell line (Ped-2E9). Actively growing live spiral cells of *Campylobacter jejuni* in physiological saline, at different doses, were challenged onto target hybridoma cells in 96-plate wells, and gentamycin was added at 0 to 3 h to stop further exposure. Significant damage to hybridoma cells was caused by challenge with live spiral cells of *C. jejuni* at MOE (*C. jejuni* cells: target hybridoma cells) ranges of 10,000:1 through 625:1. The death of target hybridoma cells due to *C. jejuni* was significantly reduced or completely arrested if gentamycin was added at 0 h, except at the highest MOE of 10,000:1 at which target hybridoma cells died. At MOE of 5000:1 or less, hybridoma cells were apparently infected by *C. jejuni* within 3 h of exposure. Our results demonstrate that all doses of spiral cells of *C. jejuni* tested, at MOE of 10,000:1 through 625:1, are capable of causing moderate to high mortality to the target hybridoma cells within 24 h (50 to 100% cell death). *C. jejuni* was recovered from internalized hybridoma cells for differentiating invasive and non-invasive *C. jejuni* strains present in raw broiler carcass rinses. Further tests are under way to confirm with a PCR assay (518-bp band) the presence of an invasive gene (fragment on iam locus) in these *C. jejuni* isolates.

P096 Comparison of Total Cost, Method Efficiency, and Laboratory Productivity of Selected Microbiological Test Kits

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Several microbiology rapid test kit methods are currently available for use in the isolation and detection of *Salmonella*, *Listeria*, and *Escherichia coli* O157:H7 from foods. Government and commerical laboratories that screen for these analytes are faced with a decision as to whether to use the standard method as outlined for detection of these organisms or to utilize one of the commercially available test kits. The objective of this study was to analyze the comparative value of five commercially available test kits (BioControl Assurance® EIA, bioMèrieux VIDAS®, Neogen REVEAL®, DuPont Qualicon BAX® automated detection system, and TECRA® Visual ImmunoAssay), as well as the standard methods for each of the test organisms (*Salmonella*, *Listeria*, and *Escherichia coli* O157:H7). A total of twenty-six collaborating laboratories participated in the study. Collaborators who routinely utilized the test methods in their laboratory were sent a questionnaire specific for each test kit (or standard method) and specific for each target organism. Information gathered from the survey included the amount of hands-on labor, total elapsed time, and cost of the materials and disposables utilized in the test method for each of the seven processing steps outlined (sample receipt and recording; sample preparation, enrichment, and screening; result analysis, confirmation and reporting). Results of the comparative study indicated that materials and disposable costs, hands-on labor, as well as the total elapsed time of the method, are all factors that need to be considered when selecting the appropriate test for a laboratory.

P097 Methods for the Recovery and Detection of Human Enteric Viruses from Complex Food Matrices

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Composite or complex foods containing plant material, animal tissue, and other ingredients may become fecally contaminated and have been epidemiologically linked to patients with infectious hepatitis and viral gastroenteritis. However, little information is available on methods for the recovery of human enteric viruses from complex foods. Experiments were conducted to recover hepatitis A virus (HAV) and Norwalk virus (NV) from strawberry sauce (SS), tomato sauce (TS), and pasta mixed with salt, sugar, and/or oil. Coliphage MS2 was used in some experiments as a surrogate measure for HAV. Methods employed for virus recovery from seeded food samples included amino acid (AA) elution, polyethylene glycol (PEG) precipitation, fluorocarbon extraction, and RNA extraction, followed by reverse-transcription polymerase chain reaction (RT-PCR) or infectivity assays. When pasta samples were subjected to AA elution, PEG precipitation and RNA extraction, HAV and NV were detectable at levels corresponding to 67 and 5 RT-PCR units (RTPCRU) / gram, respectively. Following pH adjustment and AA elution, 100% of infectious HAV and MS2 were recovered from SS and TS samples. When AA elution was combined with PEG and RNA extraction, levels of HAV corresponding to 1300 RTPCRU / mL were detectable in both SS and TS. Chemical extraction of viral RNA is required, as the presence of polysaccharides and other material following the PEG step inhibited virus detection by RT-PCR. These experiments demonstrate that low levels of human enteric viruses can be efficiently recovered and detected from complex foods with the use of simple but effective processing techniques.

P098 Evaluation of Methods for Declumping of *Mycobacterium avium* ssp. *paratuberculosis*

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the etiological and putative disease agent for Johne's disease in cattle and Crohn's disease in humans. However, research on this organism is controversial. MAP cells clump prolifically, thereby complicating results from growth and survival studies. Accurate quantitation requires an effective means to declump these cells in liquid menstrua. Various techniques were compared in duplicate, including sonication (0 to 90 min) with mild heating (50°C, 10 to 90 min), multiple passages (0 to 90 times) through a 26-guage syringe needle, agitation (Vortex™ 1 min, high speed) with microscopic (4 and 106 μm) glass beads, Octanol (3 to 250 μL per ml) and homogenization (Polytron™ at maximum speed, 1 min) alone and in combinations with heating (60°C, 3 min), and agitation treatments with glass beads. A lux transformed luminescent MAP strain was used. Luminescence readings were compared with absorbance at 540 nm, and microscopic counts of Zeihl-Neilson Carbol-Fuchsin stained cells before and after treatments. Both heating (60°C for 3 min) and agitation with glass beads was found to have a lethal effect. Octanol (5 μL per ml for 15 min) appeared more effective than homogenization, which appeared more effective than 25 passages through a 26-gauge needle. Octanol (5 μL/ml) treatments greater than 15 min resulted in cell injury. The volume and time of these techniques were standardized. These approaches, if properly used, should allow more accurate and reliable determinations of the factors influencing growth and survival of MAP.

P099 DSC Effect of Enumeration Media on the Recovery of High-pressure Processed *Bacillus subtilis* Spores

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Recovery media effects can be crucial in evaluating the microbial efficacy of a new food processing technology. Different media can yield different levels of survivors and/or lethal injury. Evidence is growing that it is possible to combine high pressure and elevated temperatures to produce pressure assisted thermal process cycles capable of yielding superior quality low acid food. The objective of this work was to evaluate the effect of commonly employed non-selective media on the recovery of high-pressure treated bacterial spores. *Bacillus subtilis* spores (10^6 CFU/mL) in deionized water were used for testing. A pilot scale high pressure processor (equipped with an internal heater) was used to process the samples over a range of pressures (400 to 700 MPa), process temperatures (70 to 90°C), and process holding time (0.01 to 180 s). The influence of recovery media (Trypticase soy agar-TSA, Nutrient agar-NA) during single and double pulse high pressure processing (HPP) was also evaluated. Samples were serially diluted and spread-plated on different recovery media and incubated at 35°C for 36 to 48 h. TSA was found to produce higher levels of recovery after single pulse HPP when compared to NA

regardless of process pressure and temperature; however, the difference in recovery seen on the two media was minimal after double pulse HPP. It is proposed that the difference in recovery on the two media after single pulse HPP is due to spore injury, i.e., NA is unable to support the recovery of a sublethaly injured spore population that is able to recover and form colonies on TSA.

P100 The Use of Immuno- and Cytotoxicity Assays in the Detection of Enterotoxins in Filtrates from Strains of *Bacillus* spp.

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Filtrates from strains of *Bacillus subtilis* and *B. cereus* were evaluated for the presence of hemolytic, non-hemolytic enterotoxins and enterotoxin K by use of commercially available immunological assays (TECRA and Oxoid). The same filtrates were further subjected to cytotoxicity tests on Vero and HEp-2 cell lines. Results from the current study demonstrated that there was no cytopathogenic effect associated with the concentrated filtrates from two strains of *B. subtilis* after 24 h of incubation. In contrast, partial destruction of the Vero cell monolayer was observed within 6 h of incubation upon the addition of concentrated filtrate from *B. cereus* ATCC 49064. Correspondingly, no reduction was seen in the incorporation of 14C-leucine by Vero cells treated with the concentrated filtrates from *B. subtilis*. However, when filtrate of *B. cereus* ATCC 49064 was used, a 100-percent inhibition in the incorporation of 14C-leucine by Vero cells was demonstrated. Vacuole production was also observed when HEp-2 cells were exposed to filtrate of *B. cereus* ATCC 49064. However, results from our vacuolation assays indicated no morphological changes when HEp-2 cells were treated with concentrated filtrates from *B. subtilis*. Finally, PCR-based methods were used to test for the presence of genes with possible enterotoxigenic capacity in all strains of *Bacillus* spp. The results were then used to establish whether the strains of *Bacillus* spp. produced the hemolytic, non-hemolytic enterotoxins and enterotoxin K under the same conditions that allowed detection for a known toxigenic strain of *B. cereus*.

P101 Efficacy of Clostridial Plate Counts as a Substitute for Botulinum Toxin Detection during Botulinal Challenge Studies of Foods

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The objective of this research was to determine if *Clostridium botulinum* or *Clostridium sporogenes* plate counts could be used as a reliable substitute for botulinum toxin detection in foods during botulinal challenge studies. Nine widely varied food systems inoculated with spores of *C. sporogenes* PA3679, proteolytic *C. botulinum*, or nonproteolytic *C. botulinum* were tested periodically during incubation for numbers of *C. botulinum* or *C. sporogenes* using Differential Reinforced Clostridial Agar (DRCA). Samples inoculated with *C. botulinum* were also tested for the presence of botulinum toxin using the standard mouse bioassay. Significant (> 2 log CFU/g) increases in proteolytic *C. botulinum* plate counts were detected during incubation in only five of nine foods tested, even though botulinum toxin was detected by the mouse bioassay during incubation in all foods inoculated with proteolytic *C. botulinum*. When foods were inoculated with *C. sporogenes* and incubated under the same conditions as those inoculated with proteolytic *C. botulinum*, significant increases in *C. sporogenes* plate counts were detected during incubation in only six out of nine foods. Significant increases in nonproteolytic *C. botulinum* plate counts were detected in only two of the five foods that supported toxin production by nonproteolytic *C. botulinum* under refrigeration or mild abuse conditions. Results of this study support the hypothesis that detectable significant growth of *C. botulinum* or *C. sporogenes* as determined by direct plating methods may not be a reliable indicator of the ability of a food to support toxin production by *C. botulinum*.

P102 3M™ Petrifilm™ Staph Express Count Plate for the Rapid Enumeration of *Staphylococcus aureus* in Foods – Collaborative Study

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This interlaboratory collaborative study evaluated and compared the AOAC INTERNATIONAL method for detection of *Staphylococcus aureus* in foods with a commercial product, the 3M™ Petrifilm™ Staph Express Count Plate and Disk, for recovery of *S. aureus* in foods. In the study, 14 food types – smoked salmon, pepperoni, cured ham, cooked and diced chicken, frozen lasagna, custard, frozen mixed vegetables, frozen hashbrowns, frozen batter coated mushrooms, raw milk, yogurt, cheese, whey powder, and ice cream – were analyzed for *S. aureus* by several collaborating laboratories. For each food tested, the collaborators received eight blind samples consisting of a control sample and three levels of inoculated sample, each in duplicate. Each sample was tested for *S. aureus* using the Petrifilm Staph Express Count plate method, as well as AOAC® Official MethodSM 975.55, the 3-plate Baird-Parker agar plus coagulase test (BPA). The

precision estimates (repeatability or within-laboratory variation, and reproducibility or between-laboratory variation) were calculated using standard statistical techniques. The mean log counts for the two methods were not statistically different for all foods and levels tested. The repeatability and reproducibility variances of the 24 h Petrifilm Staph Express Count plate method were significantly better than those of the 72 h BPA method 26% of the time and not significantly different 95% of the time.

P103 Enumerating 3M™ Petrifilm™ Aerobic Count Plates Using the PetriScan® Automated Colony Counter

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In the food and dairy industries, aerobic plate counts are determined by a time consuming and laborious hand counting method. The PetriScan automated colony counter was developed in order to improve efficiency in the microbiology laboratory. In this study, colony counts of food, dairy, and milk products plated on 3M Petrifilm Aerobic Count Plates were compared using both automated and manual count plate methods. For sample variation, 16 different food, dairy, and milk products were used. Samples were prepared and serially diluted using Butterfield's diluent according to approved AOAC methods and APHA's Standard Methods. Plates were inoculated, incubated, and counted according to AOAC methods. For data collection, plates with counts between 5 and 300 colonies were included. A total of 55 low (5 to 30), 29 medium (31 to 100), and 23 high (101 to 300) count plates were used. Duplicate results were recorded for both methods; hand counts were tallied by two scientists. The duplicates of the mean log values for manual counts varied by 0.0005 and 0.0007, and the duplicates for the automated counts varied by 0.0011. The mean log value difference between the automated and manual counts for pooled data was 0.035. The correlation coefficient for the regression line comparing the automated and manual count methods for pooled data was 0.98. The regression equation was $y = 0.9257 \times + 0.0781$. These results demonstrate that the PetriScan automated colony counter is a comparable and practical alternative to the standard method of manually counting plates.

P104 Fourier Transform Infrared Spectroscopy for Rapid Detection, Identification, and Enumeration of Bacteria in Foods

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The presence of microorganisms in food products has important ramifications for safety, quality, regulations, and public health. Rapid and reliable methods are required for the detection of microorganisms, especially foodborne pathogens. The use of Fourier transform infrared (FTIR) spectroscopy and chemometrics (multiple linear regression and hierarchical cluster analysis) for the rapid detection, identification, and enumeration of bacterial in cultures was investigated. In this study, gram-negative (*Escherichia coli* O157:H7 (H1730, F4546, Cider, E0019), *Klebsiella pneumoniae* (ATCC 4208)) and gram-positive (*Lactobacillus reuteri* (SD2112, MM2-3, MM7, CF2-7F, MF14-C)) organisms were used. Pathogens were grown in brain heart infusion agar (BHI) whereas *Lactobacillus* strains were grown in MRS broth. All strains were incubated at 37°C for 24 h. A FTIR spectrometer with attenuated total reflectance (ATR) was used to measure aqueous microbial samples. Fresh broth without microorganism added was used as background. The spectral data collection was complete in about 3 min. Different spectral regions (3700 – 2800 cm-1 and 1800 – 1000 cm-1) were used to identify and classify isolates. Bacteria were clustered into negative (*E. coli* and *K. pneumoniae*) and positive (*L. reuteri*) groups and the rate of correct classifications was 100%. Hierarchical cluster analysis demonstrated the differences between different strains of *E. coli* and *L. reuteri*. A dendrogam indicated that CF2-7F was different from the rest of *L. reuteri* and it was isolated from the infant sample while the others were from adults. Multiple linear regression was used for enumeration of bacteria, and a R^2 value was 0.999. Our results indicated that FTIR spectroscopy could be used as a rapid method for the identification and enumeration of bacteria in foods.

P105 Menadione-catalyzed Luminol Chemiluminescent Assay for the Rapid Detection and Estimation of Viable Bacteria

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This study investigated the rapid detection and estimation of viable bacterial cells by the menadione-catalyzed luminol chemiluminescent assay method. Chemiluminescent detection was performed by measuring O_2- dependent luminol intensity. Menadione is considered to be reduced by NAD(P)H: menadione-oxidoreductase in bacteria, and the active oxygen species as final products are determined by luminol chemiluminescent assay. The minimum CFU level was 2.5×10^4 CFU/well (0.1 ml), as determined using a 96 well plate, after 10 min incubation with menadione, followed by chemiluminescent assay for 2 s. Inoculation of 10^0 to 10^1 CFU/ml of *E. coli* followed by cultivation for

8 h in culture broth medium allows detection of *E. coli* by this method. By monitoring the increase of chemiluminescece intensity and by kinetics analysis, this method yielded a highly accurate standard curve. Four artificially inoculated liquid food samples (milk, vegetable juice, green tea and coffee) were evaluated as to the viable bacterial cell count by use of this method and the results revealed that inoculation of 10^0 to 10^1 CFU/ml of *E. coli* and incubation for 8 h could yield detectable results by this method. However, green tea and coffee samples required filtration before inoculation, because the influence of luminescence inhibition was high. This chemiluminescent assay method requires a few min for incubation with menadione and a few seconds for the measurement of chemiluminescece intensity. Therefore, the luminol chemiluminescent assay method could be useful for rapid determination of viable bacterial cells in food samples and could have reduced experimental cost, since this method uses a 96-well plate.

P106 A Rapid Protocol for the Isolation and Identification of Pathogens from a Lateral Flow Device

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Lateral flow devices (LFD) are a fast and convenient way to routinely screen foods for pathogens. Positive test results are presumptive and additional confirmation is needed for regulatory/management purposes. The initial step for confirmation is to obtain a pure culture, which is tedious, expensive and time consuming. The confirmation process can often be very challenging, particularly if the target culture concentration is significantly lower than that of background flora. Products are available that aid in the acquisition of a pure culture isolate from a complex sample; however, these products are generally multi-stepped and time consuming. The protocol described here is a quick and easy method for the isolation of pure cultures from enriched food samples, using the test line from the LFD. Live target cells are bound by stationary antibodies in the test zone of the LFD and subsequently concentrated in this zone. After a sample is enriched, a portion of the culture is transferred directly to the LFD and the test is performed. Following the completion of the assay, the test line is excised in a width of ca. 5 mm. The cut membrane is directly struck to various agar plates. The plates are incubated and examined for typical colonies of interest. Using this method, we have successfully isolated and confirmed *E. coli* O157 strains from beef samples while conventional methods failed; in addition, we have routinely used this method for our assay development. This is an efficient and useful method for isolation and identification of presumptive positive cultures by lateral flow tests.

P107 DSC Direct Detection of Bacterial Pathogens in Representative Dairy Products Using a Combined Bacterial Concentration – PCR Approach

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It has been suggested that the efficacy of rapid pathogen detection could be expanded if the bacteria were concentrated from the sample matrix before detection. The purpose of this study was to develop a concentration-detection strategy for two representative bacterial pathogens in a complex dairy food matrix. Eleven-gram samples of plain nonfat yogurt or mild cheddar cheese were seeded with *L. monocytogenes* or *S. enterica* serotype Enteritidis at levels of $10^2 - 10^6$ CFU per sample. Samples were then processed for bacterial concentration by use of high-speed centrifugation (9,700 × g) followed by detection with both cultural and molecular methods. Recovery efficiency was calculated based on direct plating of the retained centrifugation pellets and confirmed by enumeration of bacteria remaining in the discarded supernatants. Bacterial recoveries after centrifugation ranged from 53 – >100% and 69 – >100% for serovar Enteritidis and *L. monocytogenes*, respectively, in both product types. There were no significant differences in recovery efficiency at different inocula levels, and losses to discarded supernatants were always < 5 %, regardless of dairy product or pathogen. When followed by pathogen detection using PCR, detection limits of 10^3 and 10^2 CFU per 11 g sample were achieved for *L. monocytogenes* and serovar Enteritidis, respectively, in both product types and without prior cultural enrichment. This study represents progress toward the rapid and efficient direct detection (without cultural enrichment) of pathogens from complex food matrices at detection limits approaching those that might be anticipated in naturally contaminated products.

P108 Rapid and Simultaneous Detection of Nine Foodborne Pathogenic Bacteria Using Multiplex PCR Method

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Current methods for detection of foodborne pathogens involve the culture of samples on

selective media with a period of 4 to 7 days typically required. As an alternative, we have developed a simultaneous screening method by multiplex PCR in a single reaction tube for rapid detection of nine foodborne pathogenic bacteria. The specific primers for multiplex PCR amplification of *C. jejuni*-specific hypothetical protein, shiga-like toxin, femA, diarrheal enterotoxin, toxR, iap, ail, invA and virA genes were designed to allow simultaneous detection of *Campylobacter jejuni*, *E. coli* O157:H7, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp., and *Shiegella sonnei*, respectively. To confirm the specificity of each primer pair for the respective target gene, three types of experiments were carried out with boiled cell lysates and its DNAs, respectively. A single amplification for each primer pair was identified, with total chromosomal DNAs prepared from food samples spiked with 9 pathogenic bacteria. Using the multiplex primers, another single amplification was used to confirm with total chromosomal DNA from samples contaminated with single specific pathogenic bacteria. In multiplex PCR with mixed DNA samples, specific bands for corresponding genes were simultaneously detected in a single tube reaction. This study demonstrated that the detection of all nine human pathogenic bacteria, which is time consuming, could be accomplished in less than 24 h by use of this PCR assay. The multiplex PCR assay was highly reliable and may be used as a rapid and sensitive means of identifying pathogenic bacteria in foods.

P109 PCR-based Fluorescent Assay for Rapid Detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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A polymerase chain reaction (PCR) assay combined with a DNA fluorescence detection method was evaluated for rapid detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The bacterial cultures were serially diluted to obtain final concentrations of 10^0–10^5 CFU/ml. One ml of each sample was used to obtain the DNA template and 5 microliter of the sample template was added into 25 microliter of SYBR Green PCR Master Mix and two specific *E. coli* O157:H7 (UidAa, UidAb) or *L. monocytogenes* gene primers (FP, RP). The reaction was carried out in a thermocycler. Finally, the fluorescence signal of each PCR product was measured by use of a fluorometer. Results indicate that this test could detect as few as 1 CFU/ml of *E. coli* O157:H7 or 2 CFU/ml of *L. monocytogenes* without any enrichment. In addition, the PCR-based fluorescence method could detect the target bacteria in minutes after PCR amplification compared to hours with gel electrophoresis and also could be done earlier in PCR amplification. The method was evaluated for detection of pathogens in food samples, including poultry, meat and vegetables.

P110 Detection of Biowarfare Agents in Food by Use of Fluorescent PCR

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Following the events of 9/11/01, the threat of a biological warfare attack on the nation's food supply has become a distinct possibility. In response, we developed PCR assays to detect the presence of the Biothreat agents *Bacillus anthracis* and *Yersinia pestis* in various food matrices. Assays were developed for the *B. anthracis* virulence genes lef (lethal factor) and capA (capsule protein), and the *Y. pestis* caf1 gene (F1 antigen). Twenty-five gram samples of bread, ground beef, raw fish, shrimp, green pepper, or potato salad were inoculated with serially diluted avirulent *B. anthracis* spores or *Y. pestis* cells. Samples were enriched for 21 and 48 h and tested by PCR/melting curve analysis. Assay sensitivities varied relative to the food matrix tested. The lef, capA, and F1 assays produced positive results for bread samples inoculated with 6 *B. anthracis* spores, 5 *B. anthracis* spores, or 30 *Y. pestis* cells. For raw ground beef, the lef and capA assay produced positive results for samples inoculated with 6 and 42 *B. anthracis* spores, respectively, while the F1 assay showed no positive results for samples inoculated with as many as 73,000 *Y. pestis* cells. The poor sensitivity in certain food matrices may be attributed to a high level of background microflora, which may have outcompeted and outgrown the target bacteria. This study demonstrates that the lef, capA, and F1 assays are capable of detecting low levels of *B. anthracis* and *Y. pestis* in certain food matrices, and should prove to be of great use in food testing laboratories.

P111 A Comparative Study of Two Immunoassays for the Detection of Chloramphenicol in Milk and Shrimps

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In the US, the antibiotic Chloramphenicol (CAP) has been totally banned for use in animals kept for food production. Nevertheless, some CAP residues have been regularly isolated from different exported foods, such as shellfish. In our study, we compared two immunoassays, Ridascreen Chloramphenicol

(R-CAP) and Transia Plate Chloramphenicol (TP-CAP), routinely applicable for CAP detection in milk and shrimps. With the 10 samples of non-contaminated shrimp samples, the calculated limits of detection for the assays (mean+3 standard deviations) were 0.239 and 0.059 ppb, respectively for R-CAP and TP-CAP. When spiked with 0.1 and 0.5 ppb of CAP, the TP-CAP showed a mean recovery of 79% and 75%, and the R-CAP gave a measurable recovery of CAP for only the 0.5 ppb spiking. With the 10 blank skimmed milk samples, the calculated limits of detection for the assays were found to be 0.320 and 0.005 ppb respectively for R-CAP and TP-CAP. When spiked with 0.1 ppb, the mean recovery of the 10 milk samples was evaluated to 16% for the TP-CAP and 71% for the R-CAP, but with tremendous differences in the blank values: if, with the TP-CAP assay, all the blank milk samples offered CAP amount lower than 0.004 ppb, the R-CAP assay gave CAP amount between 0.112 and 0.257 ppb and so most of the spiked samples showed CAP concentration lower than the calculated limit of detection (0.320 ppb). The Transia Plate Chloramphenicol showed a higher sensitivity and specificity than Ridascreen Chloramphenicol for CAP screening in milk and shrimps.

P112 Rapid Determination of Histamine in Food Using a Colorimetric Enzyme Assay

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A colorimetric enzyme assay for the quantitative analysis of histamine in food has been developed using histamine dehydrogenase from *Rhizobium* sp. Histamine dehydrogenase catalyses specifically the oxidation of histamine and no other biogenic amines, such as putrescine and cadaverine. This reaction, in the presence of 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS), produces a colored tetrazolium salt which can be measured at 460 nm. The method does not require removal of substances that interfere with the assay, as the HPLC method and the AOAC method do. The method also does not require complicated procedures such as are needed in the HPLC method, AOAC method or EIA method. Using this method, we have studied the absorbances obtained using 0–300 ppm of histamine standard. In this concentration range, a positive correlation was found between histamine concentration and absorbance ($r = 1$, CV < 2.5%). Assays of canned tuna in oil and of soup with added histamine (5–100 ppm) showed very good recoveries, 96–113% and 98–108% respectively. This method was compared with four other reference methods (HPLC method, AOAC method, and 2 commercial EIA test kits). The histamine content from raw tuna and commercial canned tuna containing histamine in high concentration was determined using five methods. Equivalent results were obtained with the five methods, including our new histamine enzymatic assay. According to these results, this new enzymatic method is not only simple and fast, but also as reliable as the conventional methods. This method can be easily adapted to determine histamine in food.

P113 DSC Screening for Potential Aflatoxin-producing Molds in Korean Fermented Foods and Grains by Multiplex PCR and Enzyme Immunoassay

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A multiplex polymerase chain reaction (PCR) and ELISA were developed for the detection of potential aflatoxin-producing molds in Korean fermented foods and grains. Three genes, avfA, omt-A, and ver-1, coding for key enzymes in aflatoxin biosynthesis, were used as aflatoxin detecting target genes in multiplex PCR. DNA extracted from *Aspergillus flavus*, *A. parasiticus*, *A. oryzae*, A. niger, *A. terreus*, *Penicillum expansum* and *Fusarium moniliforme* was used as a PCR template to test specificity of the multiplex PCR assay. Positive results were achieved only with DNA that was extracted from the aflatoxigenic molds *A. flavus* and *A. parasiticus* in all three primer pairs. We compared the sensitivity of PCR in the detection of aflatoxin-producing molds DNA and that of the direct competitive enzyme-linked immunosorbent assay (DC-ELISA) in detecting aflatoxin in artificially contaminated Meju samples. The sensitivity of the PCR procedure was higher than that of DC-ELISA. The PCR assay developed in this study required only a few hours, allowing the rapid and simultaneous analysis of many samples at low cost. A total of 22 Meju samples, 24 Doenjang samples and 10 barley samples commercially obtained from Gyeognam Province of Korea were analyzed. The DC-ELISA assay for aflatoxin detection gave negative results for all samples, while the PCR-based method gave positive results for 1 of 22 Meju samples and 2 of 10 barley samples. Followed incubation of the three positive samples on malt extract agar, DC-ELISA also gave positive results for aflatoxin detection. All Doenjang samples were negative, suggesting that the contamination of aflatoxin and aflatoxin-producing molds in Doenjang is probably low.

P114 *In Vitro* Study of Ochratoxin A Production by *Aspergillus carbonarius* and *A. niger* Isolates and Detection by HPLC and Enzyme Immunoassay

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Black Aspergilli are cosmopolitan fungi, frequently detected in processed food. Recently it was found that *Aspergillus carbonarius* and some *A. niger* strains are able to produce ochratoxin A (OTA), a nephrotoxic metabolite. To examine this possibility, we investigated ten strains of *Aspergillus carbonarius* and 24 strains of *A. niger* isolates obtained from green coffee samples from Brazil, to produce OTA "in vitro" by using cracked corn (a_w 0.98, incubated at 25°C, during 12 days) as matrix. For detection and quantification of mycotoxin, two methods were used: high performance liquid chromatography (HPLC) and enzyme immunoassay (EIA). The detection limit was 1 and 0.6 µg/kg, respectively. OTA production ability was detected in 60% of *A. carbonarius* (six out of ten strains) by both methods, with levels ranging from 5 to 26 µg/kg (HPLC) and 1 to 3 µg/kg (enzyme immunoassay). Toxin production by *A. niger* was detected in 25.0% (seven out of 28 strains), in lower levels, from 0.6 to 1.1 µg/kg, when analyzed by EIA. By HPLC, no OTA was detected.

P115 DSC Efficacy of Capric/Caprylic Acid, Lactic Acid, Glycerol Monolaurate and Peroxyacid Alone or in Combination for Inactivating *Escherichia coli* O157:H7 on Artificially Contaminated Alfalfa Seeds

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Alfalfa seeds were inoculated with a 3-strain cocktail of *Escherichia coli* O157:H7 by immersion so as to contain ~ 5 to 7 log CFU/g and exposed for 1, 3 and 5 min to the following four treatments: Treatment 1–15,000 ppm capric/caprylic (CC), 15,000 ppm lactic acid (LA) and 7,500 ppm glycerol monolaurate (GM); Treatment 2–10,000 ppm CC, 10,000 ppm LA and 5,000 ppm GM; Treatment 3–3,750 ppm peroxyacid, and Treatment 4–15,000 ppm CC. Appropriate dilutions in neutralizing buffer were spiral-plated on Tryptic Soy Agar with 0.6% Yeast Extract (TSA-YE), Cefixime Tellurite Sorbitol McConkey Agar (CT-SMAC) and TSB-YE overlaid with CT-SMAC for enumerating mesophilic aerobic bacteria (MAB), *E. coli* O157:H7, and numbers of injured and non-injured *E. coli* O157:H7, respectively. Treatment 1 reduced MAB > 6.68 logs and *E. coli* O157:H7 > 5.09 logs without recovery of injured cells for all exposure durations. After 3 min, Treatment 2 reduced MAB and *E. coli* populations 3.41 and > 4.75 logs, respectively. Treatments 3 and 4 yielded maximum reductions of < 1.38 – 2.71 and 0.74 – 1.97 logs for MAB and *E. coli* O157:H7, respectively. Based on previous observations, a mixture of Treatments 1 and 3 did not significantly decrease the germination rate compared to the untreated control. Hence, it can be concluded that Treatment 1 can be used to effectively inactivate *E. coli* O157:H7 on alfalfa seeds.

P116 Applicability of Image Analysis in Modeling of Bacterial Growth

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Collecting data on microbial responses to a wide range of factors (such as pH, temperature, etc.) with plating methods is a laborious and time-consuming task. On the other hand, alternative and modern rapid techniques, such as turbidimetry and conductance, although they have been extensively studied, require in their application consideration of limitations and extrapolations for translating data into kinetic parameters. Image analysis offers an objective means for direct spatial measurements of cell or even colony size (area). Such measurements may be converted to population either through distribution functions or through correlation of image data with those from a plating method. This study attempted to compare growth rates of *Escherichia coli* O157:H7 estimated by two methods of Image Analysis: (1) by measuring bacterial cells under a microscope, (2) by measuring changes in the area of bacterial colonies on agar plates, for pH ranging from 4.0 to 7.0 and temperatures ranging from 10 to 30°C. The estimated growth rates correlated well with those from plate counts and additional kinetic data from the first two techniques were used for development of a predictive model for the growth rate of *E. coli* in response to pH and temperature. Widely used secondary models, such as Arrhenius, polynomial and square root models, were fitted to the data-set generated by the Image Analysis technique. Predictions of models were then converted into actual growth rates by use of calibration curves and compared with independent literature data to validate the model.

P117 DSC Effect of Liquid Alum on Naturally Occurring *Salmonella* and *Campylobacter* in Poultry Broiler Production Facilities

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The use of dry alum (aluminum sulfate) as a broiler litter amendment has been shown to reduce ammonia emissions, but little information is available on the application of liquid alum to poultry litter to reduce *Salmonella* and *Campylobacter*. Our objective was to test the survival of both pathogens and to enumerate them in four adjacent broiler facilities of the same design that were treated with the following rates of liquid alum: 0 L/m^2, 0.82 L/m^2 (45kg), 1.64 L/m^2 (91kg) and 2.46 L/m^2 (136kg). Each broiler house contained approximately 30,000 birds with a six-week grow-out period and approximately two weeks between harvest of birds and introduction of the next flock. Four different composite samples were taken for each sample event; the sample events for each growout occurred at the beginning of the growout and 21 days after alum application. Modified FDA and USDA methods were used to detect each bacterium in the litter samples and to enumerate pathogens. All isolates were confirmed biochemically and serologically. Liquid alum had an accumulative effect over six months in reducing *Salmonella*. For reduction of *Salmonella*, the 1.64 L/m^2 and 2.46 L/m^2 alum applications were more effective than the 0.82 L/m^2 alum application. There was a strong correlation between litter moisture content and *Salmonella* levels but not between *Salmonella* levels and pH of litter, except when litter pH was reduced below 3.5. *Campylobacter* was detected for four months during the six-month study. The 1.64 L/m^2 alum application reduced *Campylobacter* levels by 2 log CFU/ml and the 2.46 L/m^2 rate reduced *Campylobacter* levels by 3 log CFU/ml. Aerobic Plate Counts were performed, and alum had no effects over the six-month period. These research findings suggest that the use of alum can reduce *Salmonella* and *Campylobacter* over a six-month time period in a poultry broiler production facility.

P118 Genotypic Characterization by Pulsed-Field Gel Electrophoresis and Antibiotic Resistance of *Campylobacter* Strains Isolated from Poultry Litter

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Campylobacter is a major cause of gastroenteritis throughout the world, and poultry consumption is most often cited as the mode of transmission to humans. In this study, the genetic diversity of *Campylobacters* in the poultry house environment and their association with the flock is investigated. From November 2000 to August 2001, *Campylobacter* isolates were recovered from drag-swab samples taken from the litter of an Iowa poultry farm. Macrorestriction analysis of genomic DNA by pulsed field gel electrophoresis (PFGE) was used to characterize the isolates and their antibiotic resistance was determined using the E Test method. PFGE analysis of *Sma*I-digested DNA from all the isolates yielded between 5 and 11 fragments that ranged in size from about 46 to 521 kb. 84% of the isolates were assigned to one macrorestriction profile and they were distributed throughout the house during the year. PFGE analysis of *Kpn*I-digested DNA from selected isolates yielded 5–9 fragments that ranged in sized from about 50 to 450 kb. Unexpectedly, the dominant strains in the environment were identified as *Campylobacter coli*. *C. jejuni* was isolated from the environment only once and had a PFGE pattern similar to that of *C. jejuni* strains recovered from chicken ceca. The majority of the isolates displayed no antibiotic resistance to nalidixic acid, ciprofloxacin, enrofloxacin, tetracycline, erythromycin, or ampicillin. *Campylobacter* has been reported to have a tendency for rapid gentic change and to possess an unstable genome. However, this study identified a strain of *Campylobacter* coli that remained stable for several months in the poultry environment.

P119 Mycoflora and Occurrence of Aflatoxin and Fumonisin in Poultry Feeds

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Aflatoxin and fumonisin pose a serious threat to humans and domestic animals, because they are very toxic compounds that cause illness or death when feeds or foods contaminated by them are consumed. Animal feeds under adverse conditions are an optimal substrate for mold growth and potential production of aflatoxin and fumonisin. The objective of this investigation was to evaluate the mycoflora and the occurrence of aflatoxin and fumonisin B1 in poultry feeds.Total mold counts was performed using DRBC agar according to the ICMF, and the results were expressed as CFU/g. Mold colonies were identified following the taxonomic keys of Raper and Fenell (1965), Fassatiova (1986) and Samsom et al. (1995). Aflatoxin analysis was conducted using an immunoaffinity column as a clean up procedure coupled to TLC. The fumonisin analysis was performed using the method of Syndeham et al. (1992). It was observed that mold incidence in poultry feeds ranged from 10^2 to 10^3 CFU/g and the moisture level ranged from 8 to 11%. Twelve genera of molds were isolated, and the main species identified were *A. flavus*, *F. moniliforme*, *P. hirsutum*, *P. citrinum* and *E. astelodami*. Aflatoxin was not detected in the 48 lots of feeds. However, the occurrence of fumonisin B1 was 13.3%, with levels from 30 to 500 ng/g. This study demonstrated a very low incidence of aflatoxin and fumonisin and the presence in animal feeds of potentially toxinogenic mold.

P120 Prevalence of *Campylobacter* in Chicken from Pluck Shops in Trinidad

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The prevalence of *Campylobacter* in chicken sold at "Pluck Shops" in Trinidad was investigated. "Pluck shops" were classified as medium or low sale, and samples were collected from 6 counties in Trinidad. Swab samples of cloacae of birds before slaughter and of the carcass surface after processing were plated on blood-free *Campylobacter* agar base containing CCDA Selective Supplement. The plates were incubated at 42°C in 8% CO_2 for 48 h. Of the 645 samples collected from each site, 517 (80.2%) and 541 (83.9%) of the cloacal and skin samples respectively were positive for *Campylobacter* spp. The difference was not statistically significant ($P = 0.082$). *Campylobacter* was recovered from 519 (81.1%) of 640 samples taken from medium-sale shops and from 539 (82.9%) of 650 samples from low-sale shops. The difference was not statistically significant ($P = 0.392$). St. Andrew/St. David county had the highest prevalence of *Campylobacter* from the skin samples, 88.8% (71 of 80 samples) while county St. Patrick had the lowest prevalence, 81.3% (65 of 80). The difference was not statistically significant ($P = 0.184$). For cloacal samples, the highest prevalence of *Campylobacter* was detected in Nariva/Mayaro county, 83.8% (67 of 80) compared with county Caroni with the lowest prevalence of 75% (120 of 160), but the difference was not statistically significant ($P = 0.123$). The frequency of *Campylobacter* in chickens sold at "Pluck Shops" in Trinidad is high and poses a potential health risk to the consumer. The need to institute sanitary conditions at these establishments cannot be overemphasized.

P121 *Campylobacter* and *Salmonella* in Raw Chicken: Updated Baseline Figures for 2002

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The contamination of raw chicken with *Campylobacter* and *Salmonella* is an ongoing problem concerning producers, consumers and governments. During 2002, the Public Health Laboratory Service in Wales, in cooperation with twenty Local Authority Environmental Health Departments and the Food Standards Agency Wales, carried out a survey to establish baseline figures for the percentage of raw retail chicken contaminated with *Salmonella* and *Campylobacter* within Wales, a devolved part of the UK with a population of approximately three million. Seven hundred and twenty-eight fresh and frozen samples were taken between November 2001 and December 2002. Standard enrichment and plate-based methods were used for the examination of chicken carcass rinse samples. The results revealed that overall, 72% of samples were contaminated with *Campylobacter* and 8% were contaminated with *Salmonella*. In terms of overall mean values, there were no significant differences for either pathogen between fresh and frozen carcasses and between samples taken from retailers or butchers. There was definite seasonal variation in the *Campylobacter* contamination of fresh chicken, with a significant peak in June and the lowest positive rate in February and March. There was no similar peak observed in frozen samples or for *Salmonella*. It can be concluded that *Salmonella* continues to contaminate raw chickens, albeit at lower levels than in the past, whereas *Campylobacter* are present at high levels. The survey establishes Welsh baseline figures for 2002 and provides a benchmark to monitor improvements in the quality of raw chicken available to consumers in Wales.

P122 Effect of Gut Content Contamination on Broiler Carcass *Campylobacter* Counts

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Gut contents may contaminate broiler carcasses during processing. The objective of this study was to determine the effect of contamination with intestinal contents on the numbers of *Campylobacter* detected in broiler carcass rinse samples. On each of three replicate sample days, twenty-four eviscerated broiler carcasses were collected from the shackle line in a commercial processing plant. Ceca from the same flock were also collected. Contents from multiple ceca were squeezed out manually, homogenized and placed into sterile syringes fitted with needles. Broiler carcasses were cut longitudinally into contralateral halves. Paired carcass halves were divided into three groups of eight each; then cecal contents (either 10, 50 or 100 mg) were placed onto one randomly selected half of each carcass. The corresponding half of the same broiler carcass was left uncontaminated for comparison. *Campylobacter* counts from carcass halves with cecal contamination were compared to the uncontaminated halves of the same carcasses using a paired *t*-test. In all cases, carcass halves with cecal contamination had significantly more *Campylobacter* than those without ($P < 0.01$). Carcass halves contaminated with only 10 mg of cecal contents had an average of 4.6 $\log_{10}$ CFU *Campylobacter* per ml of rinse while corresponding uncontaminated

carcass halves had 2.9 $\log_{10}$ CFU *Campylobacter* per ml rinse. These data show that even small (10 mg) spots of cecal contents can significantly increase the numbers of *Campylobacter* on eviscerated broiler carcasses. Therefore, it is important to keep such contamination to a minimum during processing.

P123 Characterization of Aerobically Growing *Campylobacter jejuni* IC 21 Isolated from Chicken Carcasses

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Campylobacter is currently becoming one of the most prominent foodborne pathogens. They have adaptation ability in response to external stresses such as oxygen, pH, temperature and nutrient starvation, through morphological changes from spiral-bacilli to the coccoid form. In this research, *Campylobacter* was isolated aerobically from chicken carcasses in local markets. The aerobically growing isolate was identified as *C. jejuni* IC 21 by morphological, physiological and biochemical identification, including 16S rRNA sequence analysis. *C. jejuni* IC 21 grew well in the aerobic condition compared with microaerophilic *Campylobacter*. It had higher oxidoreductase activities than *Campylobacter* even though there were no differences in fatty acid composition and cell surface hydrophobicity. Its catalase, superoxide dismutase and NADH oxidase activities were two to ten times higher than those of the type strain. Therefore, when *Campylobacter* such as *C. jejuni* IC 21 are exposed to the aerobic condition, adaptation growth by increase of oxidoreductase activities without morphological changes might occur, which could be detrimental in terms of food safety.

P124 DSC Influence of Dietary Vitamin E on Behavior of *Listeria monocytogenes* and Color Stability in Ground Turkey Meat following Electron Beam Irradiation

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There is growing concern that the free radical scavenging effect of antioxidants added to meats might reduce the antimicrobial effectiveness of ionizing radiation. A study was conducted to determine the effect of vitamin E on the behavior of *Listeria monocytogenes* and color stability in turkey meat following electron beam irradiation. Raw ground turkey breast meat from birds fed diets containing 0 (control), 50, 100, and 200 IU of vitamin E per kg was inoculated with a 5-strain mixture of *L. monocytogenes* to give ~10^7 CFU/g. Inoculated samples were irradiated at 0, 0.5, 1, and 2 kGy, and stored aerobically (12 days) or under vacuum (42 days) at 4°C. *L. monocytogenes* survivors were determined by plating diluted samples on Modified Oxford Medium (MOX) and counting bacterial colonies on MOX plates after 48 h at 35°C. Meat color was measured by use of a Hunter Lab colorimeter. Irradiation at 2.0 kGy resulted in 4.0 log reduction of initial numbers of *L. monocytogenes*. There were no significant differences in D-values for *L. monocytogenes* in meat irrespective of vitamin E treatment ($P > 0.05$). Also, vitamin E treatments did not affect growth rate of the pathogen in aerobic or vacuum-packaged samples following irradiation ($P > 0.05$). Compared to controls, irradiated meat from birds fed 100 or 200 IU vitamin E demonstrated significant improvement in color stability (Hunter "L" and "a" values) during storage ($P < 0.05$). Dietary vitamin E (100 to 200 IU) has good potential for improving the color stability of refrigerated turkey meat without compromising the microbial safety of the irradiated product.

P125 DSC A Longitudinal Analysis of *Campylobacter* Colonization in Sibling Turkey Flocks with Marked Differences in Colonization by *Campylobacter*

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Campylobacter spp. are currently the leading bacterial cause of acute gastroenteritis in industrialized countries. *Campylobacter* has high prevalence in flocks of commercial poultry (broilers and turkeys), and consistent production of *Campylobacter*-free flocks has not been documented. The focus of this study was to conduct a longitudinal analysis of *Campylobacter* colonization in two pairs of sibling turkey flocks (4 flocks total). In each case, one member of the pair was grown in a commercial farm, whereas the other was grown in an instructional demonstration unit within a 60 mile radius. The hatchery source for the birds, time of placement, feed regimens, and bird density were the same among the members of each pair. At the completion of the production cycle, the birds of each flock were processed in commercial processing plants, and the same feed withdrawal and transport protocol was applied to both. Both flocks grown at the commercial farms became colonized by *Campylobacter* by week 2–3, and remained colonized until processing. A large fraction (> 71%) of the *Campylobacter* isolates from these flocks were resistant to several antibiotics, including the fluoroquinolones. In contrast, *Campylobacter* could

not be isolated from either of the instructional unit flocks, at any time of the production cycle. Evaluation of the operating procedures in each farm pair suggested that husbandry features such as proper litter maintenance and meticulous use of footbaths in the instructional unit farms may be among the leading factors responsible for the absence of *Campylobacter* in the *Campylobacter*-free flocks.

P126 The Potential for Retail Poultry Packs to be a Source of *Campylobacter* or *Salmonella* Infection

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Concerns have been raised in the literature about the amount of leakage that occurs from packs of fresh poultry sold at retail. If this liquid contains pathogens, there is a risk of cross-contamination via hands or ready-to-eat foods to customers or to staff handling packs. We know from previous studies that 60% of raw poultry in New Zealand can contain *Campylobacter* and 20% can contain *Salmonella*. External surfaces of 300 retail packs of fresh poultry were tested for presence/absence and counts of *Salmonella* and *Campylobacter*. Of the samples tested, one (0.3%) was contaminated with *Salmonella* Tennessee and 72 (24%) with *Campylobacter jejuni*. However, most counts were low. *Salmonella* was found at < 0.01 MPN/cm^2. Twenty-two percent of pack surfaces were positive for *Campylobacter* but at < 1 MPN/cm^2, and 2% contained > 1 MPN/cm^2. The highest *Campylobacter* count recorded was > 3.02 MPN/cm^2. Packs of poultry offal had the highest rate of contamination (52%) followed by whole birds (34%) and portions (14.5%). The extent of leakage from each pack was qualitatively estimated and correlated with the amount of surface contamination. Where absorbent pads were used to line trays, these were not effective at controlling external contamination. These observations suggest external packs may be a significant source of cross-contamination; however, the contribution of this contamination pathway to foodborne illness can only be properly determined by development of a validated risk assessment model.

P127 Strain Persistence and Fluctuation of *Campylobacter coli* Colonizing Turkeys Over Successive Production Cycles

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The dynamics of colonization of turkeys by thermophilic *Campylobacters* is poorly understood. In this study we monitored cecal colonization of turkeys by *Campylobacter coli* over three successive growth cycles at the same farm. *Campylobacter* isolated from the ceca was predominantly *C. coli* in all three flocks. Ninety-nine percent, 3.6%, and 5.5% of the birds were cecally colonized by *C. coli*, *C. jejuni*, and both *C. coli* and *C. jejuni*, respectively. Although a total of six distinct strain types were identified by restriction fragment length polymorphism analysis of the flagellin gene flaA, two related strain types which were resistant to multiple antibiotics predominated throughout the three production cycles and were isolated from 65 of the 69 *C. coli* isolates. The relative abundance of each of these two predominant types varied significantly from one flock to the next. The data suggest that multi-drug resistant strains of *C. coli* can be recovered from turkeys over successive production cycles. The repeated isolation of these strains from successive flocks likely reflects their persistence in currently unknown reservoir(s) in the production environment or repeated introduction events, followed by establishment of these strains in response to selection pressures in the production environment.

P128 Effect of Freezing on the Survival of Cold-stressed *Campylobacter jejuni* in Ground Chicken and Chicken Skin

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Campylobacter jejuni is a major cause of bacterial enteritis in humans in the United States. Foods of animal origin, mainly poultry and poultry products, are associated with *C. jejuni* infection. While *C. jejuni* is prevalent in chickens, relatively little is known of the ability of this organism to adapt to environmental conditions, such as cold stress, relevant to poultry and poultry products. Therefore, the effect of cold stress on the survival of *C. jejuni* was examined. Ten-g portions of ground chicken and chicken skin were irradiated and artificially contaminated with 10^8 CFU/g of a cocktail of three strains of *C. jejuni*. The samples were held at 4°C for 0, 1, 3, and 7 days and then kept frozen at -20°C for 0, 1, 3, 7, and 14 days. At various time intervals, samples were removed from the freezer, 90 ml of 0.1% peptone water was added, and the samples were pummeled in a Stomacher Lab Blender for 2 min. Serial dilutions of the samples were surface plated onto modified charcoal cefoperazone deoxycholate agar (CCDA) and tryptic soy agar (TSAB) with 5% sheep blood to enumerate surviving *C. jejuni*. The CCDA and TSAB plates were incubated in sealed jars under microaerobic conditions generated by CamyPak Plus gas generators for 48 h at 42°C. The decline

of *C. jejuni* ranged from 1.4 to 3.1 $\log_{10}$ CFU/g on chicken skin and 0.6 to 1.3 $\log_{10}$ CFU/g in ground chicken after exposure at minus 20°C from 1 to 14 days. Thus, survival was greater in ground chicken than in chicken skin. Refrigeration storage produced a maximum reduction of 0.6 $\log_{10}$ CFU/g in chicken skin and ground chicken after 7 days of exposure. This research furthers the understanding of the factors affecting the survival of *Campylobacter* exposed to cold stress in foods.

P129 Incidence of *Bacillus cereus* in Retail Poultry Products

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Five chicken meat products, obtained at retail stores, were evaluated for the presence of *Bacillus cereus*. Products tested were: Breaded, fully cooked, frozen nuggets (NUGGETS); breaded, fully cooked frozen, tenders (TENDERS); fully-cooked, frozen, white meat fajita-style strips (STRIPS); raw, refrigerated boneless skinless marinated breast (FILLETS); and, raw, refrigerated, cut-up, tray pack parts (PARTS). Twenty-five g of tissue was excised from each of four replicate containers of each of the products, in each of three replicate trials (n=60). Each sample was enriched in 225 ml trypticase soy-polymixin broth for 18–24 h at 30°C, then plated on MYP agar and incubated 18–24 h at 30°C. Colonies characteristic of *B. cereus* were chosen and replated for isolation on MYP agar. Suspect colonies were confirmed as *B. cereus* by gram reaction, hemolysis on blood agar, and a biochemical test strip. Overall, *B. cereus* was detected in 27 of 60 samples. Incidence by product was: NUGGETS (11/12); TENDERS (8/12); STRIPS (6/12); PARTS (2/12); and, FILLETS (0/12). Results indicate that *Bacillus cereus* organisms were present on most retail poultry products tested in this study, especially fully cooked products.

P130 Inhibitory Effects of Organic Acid Salts on Growth of *Clostridium perfringens* from Spore Inocula during Chilling of Marinated Ground Turkey Breast

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Inhibition of *Clostridium perfringens* germination and outgrowth by salts of organic acids was evaluated during continuous chilling of ground turkey. Turkey breast meat was injected with a brine containing NaCl, potato starch and potassium tetra pyrophosphate to yield final in-product concentrations of 0.85, 0.25 and 0.20%, respectively. The meat was ground, mixed with either sodium lactate (1, 2, 3 or 4%), sodium acetate (1 or 2%), buffered sodium citrate (Ional™, 1%) or buffered sodium citrate supplemented with sodium diacetate (Ional Plus™, 1%), in addition to a control that did not contain added antimicrobials. Each product was mixed with a three-strain *C. perfringens* spore cocktail to obtain final spore concentrations of ca. 2.8 $\log_{10}$ spores/g. Inoculated products (10 g) were packaged into cook-in-bags (2 in × 3 in), vacuum sealed, cooked at 60°C for 1 h, and subsequently chilled from 54.4°C to 7.2°C in 15, 18 and 21 h following exponential chilling rates. Incorporation of sodium lactate (1%), sodium acetate (1%), Ional or Ional Plus (1%) substantially inhibited germination and outgrowth of *C. perfringens* spores compared to controls. Final *C. perfringens* total populations of 3.12, 3.10, 2.38 and 2.92, respectively, were observed following a 15 h exponential chill rate. Ional at 1% concentration was effective in inhibiting germination and outgrowth to < 1.0 $\log_{10}$ CFU/g of *C. perfringens* for all three chill rates (15, 18, and 21 h) tested. Use of sodium salts of organic acids in formulation of meat products can reduce the risk of *C. perfringens* outgrowth during chilling.

P131 *Aeromonas* spp. Associated with Commercial Poultry Processing

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Trials were conducted to examine the effect of commercial processing and refrigerated storage on *Aeromonas* bacteria on broiler carcasses. Bacteria from prescalded, picked, prechilled, chilled, and refrigerated carcasses were enumerated on Iron Agar and identified with the MIDI Sherlock Microbial Identification System. Dendrograms of fatty acid profiles of isolates were prepared to determine the degree of relatedness between *Aeromonas* isolates. Findings indicated that scalding and picking operations may significantly increase the population of *Aeromonas* spp. and other H_2S producing bacteria on broiler carcasses, but the number of these bacteria on carcasses is significantly reduced by evisceration and immersion chilling operations. The population of *Aeromonas* spp. and other H_2S producing bacteria increased during refrigerated storage of the carcasses. Dendrograms of the fatty acid profiles of *Aeromonas* isolates indicated that the same *Aeromonas* strain may be isolated from carcasses taken from different locations on the same processing line. Furthermore, the same strain may be isolated from carcasses processed at the same facility on different processing days. *Aeromonas* spp. isolated from the carcasses included *Aeromonas caviae, Aeromonas hydrophila, Aeromonas salmonicida-masoucida,* and *Aeromonas schubertii.* These experiments indicated that mechanical pickers may be a source of carcass contamination by *Aeromonas* spp. and that *Aero-*

monas bacteria that survive processing may proliferate as a portion of the carcass' pyschrotrophic spoilage microflora during refrigeration. The findings of this study provide data that may be useful in designing methods to reduce the spread of spoilage bacteria during processing and increase the shelf life of fresh poultry.

P132 Identification of Enterobacteriaceae from Washed and Unwashed Commercial Shell Eggs

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Recently, little attention has been given to the microbiology of commercial processed eggs. Therefore, to evaluate the effect of processing on the safety and quality of retail shell eggs, a storage study was conducted with unwashed and commercially washed eggs. For each of three repetitions, shell eggs were purchased from a retail processing plant, transported back to the laboratory, and stored at 4°C. Once a week for six weeks, twelve eggs for each treatment (washed or unwashed controls) were rinsed in phosphate buffered saline. In addition, three composite samples consisting of the contents of three eggs were collected. A 1 ml aliquot of each sample was then plated on violet red bile agar with overlay and incubated at 37°C for 24 h. Following incubation, plates were observed for colonies presumptive for Enterobacteriaceae. A maximum of ten isolates per positive sample were streaked for isolation before being identified to genus and/or species level using biochemical testing. Although most of the isolates from the unwashed control eggs belonged to the genera *Escherichia* or *Enterobacter*, many other genera and species were identified. These included *Citrobacter*, *Klebsiella*, *Kluyvera*, *Pantoea*, *Providencia*, *Proteus*, *Rahnella*, *Serratia*, and *Salmonella*. Non-enterobacteriaceae also recovered from the egg samples included *Aeromonas*, *Burkholderia*, *Pseudumonas*, and *Vibrio*. Very few washed egg samples were found to be contaminated with these bacteria. These data provide useful information on the effect of processing on the microbiology of commercial shell eggs.

P133 Can *Salmonella* Enteritidis Breach the Vitelline Membrane of Fresh Chicken Eggs?

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This study determined if *Salmonella* Enteritidis (SE) can breach the vitelline membrane and enter the yolk. It involved a protocol and an in vitro environment that simulated the in ovo environment, but allowed for precise SE inoculation on the vitelline membrane and independent, aseptic, non-destructive sampling of the yolk as well. The growth of SE on the membrane was followed in fresh eggs for two days at 4, 8, 15, 27 and 37°C, using three replicates for each temperature. A single strain of SE (ATCC #13076) or a cocktail of five outbreak strains of SE was used. The growth behavior on the vitelline membrane was compared to SE growth in yolk and albumen, performed in the same environment. The comparisons involving a single SE strain indicated that at 4 and 8°C, SE growth on the vitelline membrane was the same as that in albumen. However, at 27 and 37°C, its growth on the membrane was the same as that in yolk. At 15°C, its growth on the membrane was intermediate between that seen in the yolk and albumen. The 5 strain SE cocktail growth on the vitelline membrane was similar to that of the single strain under the same conditions. Most importantly, yolk samples from the 30 experiments involving single and multi-strain SE growth on the vitelline membrane showed no SE. Thus, the membrane appears to be a barrier to SE itself, but may not be a barrier to yolk nutrients that could sustain SE.

P134 DSC Hygiene and Food Safety Controls in On-farm Dairies

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A study was undertaken to ascertain the effectiveness of the HACCP and Good Manufacturing Practices in hygienic milk production on small "on-farm" dairy processors. A range of methods were employed, including self reporting questionnaires; farm audits; and microbiological and chemical analysis of premises and products by means of both traditional and rapid methods. This links closely to the protocol developed for this study of a method for auditing HACCP plans utilizing more "real time" contols. Results from the self reporting questionnaires, completed by the on-farm processors, suggested that a high level of process control and documentation was being maintained. However, this conflicted with the results of the farm audits, which found several areas of concern for product safety. Analysis of samples taken from the farms also highlighted some potential problems, including high coliform counts and a phosphatase failure. A sample of milk also tested positive for sulfamethazine. Given the high-risk nature of the foods and the fact that around 20% of product from the farms participating in this study is sold unpasteurized, this sector of the industry would benefit from some improvement. On-farm processors should be encouraged to take more responsibility and a more proactive approach to food safety. This approach, coupled with some stricter enforcements should result in higher standards and correspondingly safer products. The research will be

presented by discussing the various phases of the study, the methods employed, and the results obtained and their implication for safe milk production and consumption.

P135 Comparative Studies on Milk Discard Period of Two Ceftiofur Products: Accent™ and Naxcel™ — by TTC and Charm II Beta-lactam Receptor Assay

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Ceftiofur is one of the cephalosporin ß-lactam antibiotics which is widely used by veterinary clinics to prevent and treat respiratory infections such as pasteurellosis in cattle, swine and poultry. Ceftiofur is metabolized to desfuroylceftiofur derivatives and rapidly excreted in the milk of cows. MRL of ceftiofur is 100 ppb as desfuroylceftiofur in milk and milk discard time is not established in Korea and USA because the metabolite is excreted in amounts as low as 100 ppb within 12 h after administration. The metabolite, desfuroylceftiofur, is very unstable but has antibiotic potency so that it can be detected by TTC and a microbial receptor assay. A few scientists have reported use of HPLC and LC-MS methods to determine the metabolite residue in milk. Antibiotic positive reactions have frequently occurred in milk of cows treated with ceftiofur despite establishing no milk discard time. In these experiments, two ceftiofur products, Accent™ (LGCI) and Naxcel™ (Upjohn) were administered intramuscularly to healthy and mastitic milking cows. Milk samples were collected periodically and subjected to TTC and a receptor assay to compare the deletion profiles of 2 ceftiofur products in healthy and mastitic cows. According to our studies, the sensitivity of the TTC test and receptor assay to ceftiofur and desfuroylceftiofur was too high compared to the MRL of ceftiofur in milk. Therefore, the level of ceftiofur and its metabolites in milk would be miscalculated if the TTC and receptor assay were adopted as screening tests. The excretion profiles of ceftiofur and its metabolites are not affected by mastitis and there is no difference in the excretion profile between the two ceftiofur products.

P136 DSC Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Milk by Caprylic Acid and Monocaprylin

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Escherichia coli O157:H7 and *Listeria monocytogenes* are major foodborne pathogens in the United States. Milk and dairy products have been linked to outbreaks of these pathogens. Although a number of free fatty acids and their esters exhibit antimicrobial activity against bacteria, their effectiveness in inhibiting pathogens in food-based systems is minimal. The objective of this study was to determine the antibacterial effect of caprylic acid (CA, C8:0) and monocaprylin (MC) on *E. coli* O157:H7 and *L. monocytogenes* in milk. A five-strain mixture of *E. coli* O157:H7 or *L. monocytogenes* was inoculated in autoclaved milk (4% fat) (10^6 CFU/ml) containing 0, 25, or 50 mM CA or MC. The samples were incubated at 37, 8 or 4°C for 0, 6, 24, and 48 h. The population of surviving pathogens was determined by plating on Tryptic soy agar with incubation at 37°C for 24 h. At all the temperatures, control milk samples had a significantly higher ($P < 0.05$) population of the pathogens than samples containing the lipids, except 25 mM CA. At 37°C, both pathogens were completely inactivated at 12 h by 25 mM MC and 50 mM CA. At 4 and 8°C, MC at 50 mM concentration reduced *L. monocytogenes* and *E. coli* O157:H7 by 5.0 and 4.0 log CFU/ml, respectively, at the end of 48 h. Results indicate that MC could potentially be used to inhibit *L. monocytogenes* and *E. coli* O157:H7 in milk and dairy products, but sensory studies need to be conducted before recommending their use.

P137 Listeriosis Outbreak in Québec, Canada, Linked to Heat-treated Cheeses

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In September 2002, the Québec Public Health Laboratory (LSPQ) reported 7 cases of *Listeria monocytogenes* PFGE pattern 85 infection in the Province of Québec over a 3 month period. Listeriosis is a rare disease in Québec. This is the first time the pulsovar has been identified since laboratory supervision began 4 years earlier. The cases were divided among 4 Québec regions. An epidemiological investigation of the cases was conducted using a standardized questionnaire. All cases of *L. monocytogenes* pulsovar 85 infection reported between June and September 2002 were investigated. All cases of *L. monocytogenes* infection were investigated (without awaiting the pulsovar result) after September 13 in order to cut investigation time and attempt to limit information bias. From April to November 2002, the LSPQ received 51 strains of *L. monocytogenes*, including 17 of pulsovar 85. The other pulsovars varied widely. Three newborns were affected by the disease, and two pregnant women gave birth prematurely. The cases were divided among 7 Québec regions. Environmental investigation pointed to a heat-treated cheese as the potential source of contamination. That is the first outbreak of listeriosis in Quebec. The contamination of raw milk cheese by *Listeria* is well

known in the literature as a potential source of outbreak due to this pathogen. Thermolysis is different from pasteurization. Should heat-treated cheeses be considered raw milk cheeses?

P138 DSC Survival of *Salmonella* and *Listeria monocytogenes* on Shredded Cheese

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The behavior of *Salmonella* and *Listeria monocytogenes* (LM) was evaluated during the storage of shredded natural Mozzarella and Cheddar cheeses at refrigeration and abuse temperatures. Commercial products, with (Nat+) and without (Nat-) natamycin, were inoculated with either 5 $\log_{10}$ CFU/g of *Salmonella*, or 4 $\log_{10}$ CFU/g of LM. For each treatment, 25 g portions of inoculated cheeses were either vacuum-packaged or packaged under 60% N_2/ 40% CO_2 (MAP) and stored at 4, 10, and 15°C. Triplicate samples of each treatment were assayed at 0, 7, 14, 21, 28, 60, and 90 days for populations of *Salmonella* and LM by plating serial dilutions on XLD and Modified Oxford agars, respectively. Additionally, uninoculated duplicate samples were assayed for moisture, pH, and a_w. Moisture and a_w were similar between the Cheddar cheeses tested, but the pH of the Cheddar with natamycin was approximately 0.3 pH units lower than Cheddar without natamycin. Similar trends were observed for the Mozzarella cheeses. Average populations of *Salmonella* and LM decreased or remained constant in all treatments during the 90-day sampling period. The greatest decrease was observed in certain cheeses stored at 15°C under MAP; *Salmonella* decreased 4 $\log_{10}$ CFU/g in Nat+ Mozzarella and LM decreased 2.7 $\log_{10}$ CFU/g in Nat- Cheddar. However, populations of LM increased slightly (0.9 $\log_{10}$ CFU/g) at 2-3 weeks storage in one lot of vacuum-packaged Nat- Mozzarella incubated at 15°C, before declining. These data verify that neither cheese type supports rapid growth of *Salmonella* or LM. Additional studies are needed to determine factors that contribute to variation in pathogen survival at abuse temperatures.

P139 DSC Survival of *Listeria monocytogenes* in Vanilla-flavored Soy and Dairy Products Stored at 8°C

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The survival of *Listeria monocytogenes* strain V37 in vanilla flavored yogurt (low-fat and non-fat) and soymilk (low-fat and Plus) stored at 8°C for 30 days was investigated. Commercial samples of yogurt and soymilk were used. These samples were inoculated with either 10^4 or 10^7 CFU/ml of *L. monocytogenes*. Sampling was carried out every 3–4 days initially and then weekly for a total storage time of 30 days. The pH of the samples was measured at each sampling. After 30 days, 10^4 CFU/ml inoculated low-fat plain, vanilla and non-fat plain yogurt samples had a 2.5 $\log_{10}$ reduction and non-fat vanilla yogurt had 3.5 $\log_{10}$ reduction in the viable cell population. In yogurt inoculated with 10^7 CFU/ml, there was a 2.5 $\log_{10}$ CFU/ml reduction in plain low-fat and non-fat yogurt, and a 5 $\log_{10}$ CFU/ml reduction in vanilla flavored low-fat and non-fat yogurt. In vanilla flavored, plain low-fat and Plus soymilk samples, numbers increased from 10^4 and 10^7 CFU/ml to 10^9 CFU/ml after 7 and 3 days of incubation, respectively, at 8°C. Coagulation was observed in soymilk samples when the cell population reached 10^9 CFU/ml. In soymilk the organism did not show any change in the population up to 30 days. Vanilla seems to have an inhibitory effect against *L. monocytogenes* in yogurt but not in soymilk.

P140 Viability of Bifidobacteria in Yogurt Products

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Today, approximately 30–50% of refrigerated yogurt products in the US contain viable bifidobacteria and *Lactobacillus acidophilus*. However, during processing and storage, the number of viable cells tends to decline because of exposure to severe conditions such as oxygen and acid. The purpose of this work was to investigate the viability of bifidobacteria in yogurt products sold in North Carolina. Fifty-eight commercial yogurt products, which claimed to include bifidobacteria, were obtained from local stores in Greensboro. These products were tested for the presence of bifidobacteria and probiotic properties including antimicrobial ability and ß-galactosidase activity. Experiments were performed within 24 h of purchase. *Lactobacillus* MRS and G-M17 were used for the enumeration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, respectively. Modified BIM-25 was used for the enumeration of bifidobacteria. Our results showed that the bacterial counts ranged from 5.0 to 9.9 log CFU/ml and from 6.6 to 9.5 log CFU/ml, for *Streptococcus* and *Lactobacillus*, respectively. Bifidobacteria counts were variable, ranging from 0.0 to 5.0 log CFU/ml. Of the 58 products tested only 44 (76%) contained viable cultures. Products made by only two manufacturers contained suffi-

ciently high levels of bifidobacteria to provide health benefits. The ß-galactosidase activity for bifidobacteria isolates ranged from 0 to 925 Miller units. Few isolates showed antimicrobial activity against *Escherichia coli* O157:H7. Results obtained from this research can be used by the industry to develop new technologies to ensure that consumers receive high quality products. Findings of this study can also be used to help consumers become more aware of manufacturer's claims and demand that products live up to any claims of health benefits.

P141 DSC Geographic Information System and Epidemiological Associations among Foodborne Pathogens at the Farm

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Epidemiological data suggest that our most important foodborne hazards are *Salmonella*, *Campylobacter jejuni*, and *E. coli* O157:H7. *Salmonella*, *Campylobacter jejuni*, and *E. coli* O157:H7 all share the common characteristic of having an animal reservoir from which they spread to humans; therefore, risk reductions at every point from farm to table are necessary. Geographic Information System (GIS), a computer mapping and analysis technology, has emerged as an innovative epidemiological tool in a variety of disciplines. This study utilized GIS and Riboprinting methodology to examine relationships that exist between animals and their environments. The experimental design included 321 dairy cows and calves in a vertically integrated production system. Samples were collected monthly for 12 months from dairy cows, calves, and farm environments. A total of 12,240 samples were analyzed for *Salmonella*, *C. jejuni*, and *E. coli* O157:H7, using modifications of BAM enrichments and confirmation protocols, previously validated for each sample type. Surface water adjacent to the farm was evaluated for the presence of *Salmonella*, *C. jejuni*, *E. coli* O157:H7, and fecal coliforms, by use of the membrane filtration method and Most Probable Numbers (MPN) technique. *Salmonella* was isolated at greatest frequency during summer months (54%). *C. jejuni* was isolated with increased frequency during the winter (33-35%). The overall prevalence of *Salmonella* spp. recovered from the Tennessee River (33%) paralleled that of *Salmonella* recovered from dairy farm animal and environmental samples (33%). The most frequently isolated *Salmonella* serotypes were Senftenberg (46%), Havana (24%), and Typhimurium (20%). *Salmonella* ser. Havana was the only serotype, showing similar riboprint patterns, isolated from the farm animal, environment, and surface water samples. Feeds, insects, and bird droppings were key sources of *Salmonella* ser. Havana at the farm. Therefore, we conclude that elimination of *Salmonella* from feed and control of insects and wild birds at the farm could reduce transmission of *Salmonella* in dairy cows and dairy cow environments.

P142 Shiga Toxin-producing *Escherichia coli* in Beef Heifers Grazing Rangeland Forages

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Escherichia coli O157:H7 has been the major cause of human illness outbreaks in the United States, mainly because of consumption of contaminated beef. Worldwide, however, other shiga toxin-producing *E. coli* (STEC) have been responsible for outbreaks of human illnesses. The objective of this study was to examine prevalence of sorbitol-negative STEC in beef heifers grazing rangeland forages over the four seasons of 1999. Because of the loss of heifers and vegetation to wild fires during the summer, the study was terminated and results of the winter (February) and spring (May) were reported. Representative numbers of heifers (22 in the winter and 23 in the spring) were randomly selected from a herd of 70 for fecal sampling. Of these heifers, nine were sampled in both seasons. A random sample (n = 115) of potential STEC isolates were screened for presence and expression of the toxin genes (i.e., ST1 and ST2). Fifty-six STEC isolates were detected in feces of five heifers in the winter and two heifers in the spring, with prevalence rates of 22.7 and 8.7%, respectively. Of these isolates, 37 had and expressed the ST1 gene, 14 had and expressed the ST2 gene, and five had but did not express the ST2 gene. Only non-O157:H7 STEC (i.e., O118:H- [nonmotile] and O138:H-) were detected. Because *E. coli* O118:H- has been known to cause human illnesses, it is important to screen cattle for STEC in general to identify those carrying the risk of beef contamination with such pathogens.

P143 Supplementing Feedlot Cattle Diets with Whole Cottonseed to Decrease the Prevalence of *Escherichia coli* O157:H7

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The objective of this study was to determine whether supplementing feedlot cattle diets with Whole Cottonseed (WCS) decreases the percentage of cattle testing positive for *E. coli* O157:H7. Twenty-four crossbred beef steers (approximately

28 to 56 days before slaughter) were used in the study. Half of the steers were assigned randomly to receive a standard steam-flaked corn-based diet, and half were fed a diet that contained 15% whole cottonseed. Fecal grab samples were collected directly from the rectum of each animal and cultured for *E. coli* O157:H7 on days 0, 14 and 28 after initiation of feeding WCS. Samples were pre-enriched in GN-VCC broth and subjected to immunomagnetic separation to detect the presence of *E. coli* O157. Final confirmation was conducted using biochemical and genetic analyses. No differences in the prevalence of *E. coli* O157:H7 were detected on days 0 or 14 between the two treatment groups. On day 0, 40% and 50% of the control and treated animals, respectively, tested positive for the pathogen. Whole cottonseed had no apparent effect on the amount of *E. coli* O157:H7 detected after 14 days of feeding, with 75% and 66% of the control animals and treated animals testing positive, respectively. On day 28, differences were detected between the two groups; 41% of the control animals tested positive for *E. coli* O157:H7, whereas only 8% of the steers fed the whole cottonseed tested positive. This study adds support to previous epidemiological studies indicating that the inclusion of whole cottonseed in cattle diets is associated with lower prevalence of *E. coli* O157:H7 in live animals.

P144 Experimental and Field Evaluation of Excision and Swab-based Sampling Methods for Porcine, Ovine and Bovine Carcasses

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Bacterial recovery by excision was superior to that obtained by a swabbing method. Different sizes of the sampled areas did not have statistically significant effects on recovery, but a larger sampling area may have other advantages. Various inherent factors can reduce bacterial recovery by swabbing, and/or increase its variability, including: a) A proportion of bacteria remain un-picked on the swabbed area; b) A proportion of bacteria are reversibly transferred from the swab to the carcass during repeated strokes, and c) A proportion of bacteria remain unreleased on the swab after swab-diluent homogenization. Over 1,000 carcasses were sampled in 22 different commercial UK abattoirs at 31 separate plant visits and analyzed for TVC and *Enterobacteriaceae*. Exhaustive statistical analyses revealed that there is not a direct linear relationship between samples collected by swabbing and those collected by excision. Similar results were determined independently in other EU member states. A linear relationship between swabbing and excision was confounded by excessive variation in microbial levels on carcass surfaces for all 3 species. Variation was apparent even between adjacent carcasses in all plant processing lines sampled. *Enterobacteriaceae* were rarely encountered on carcass surfaces in plants in Great Britain. Results of a longitudinal study over 13 weeks at 3 different cattle slaughterhouses revealed that swabbing was less sensitive than excision in detecting control point failure. However, the trend analysis curves generated by swabbing were less "noisy" than those generated by excision and may therefore give a better indication of long-term plant hygiene levels.

P145 Heat Resistance of Inoculated *Salmonella* on Fresh Beef as Affected by Decontamination Treatments, Storage Temperature and Storage Time

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Although carcass decontamination technologies are used effectively, concerns have been expressed as to whether they may allow surviving pathogens to adapt and potentially become resistant to subsequent hurdles. Thus, this study investigated the effects of meat decontamination, storage temperature and time on heat resistance of *Salmonella*. Beef round pieces (2.5 × 5 × 1 cm) were inoculated (5 log CFU/cm^2 of a five-strain mixture) and subsequently immersed (30 sec) in hot water (HW; 75°C) or 2% lactic acid (LA; 55°C), or left untreated (C), and then stored aerobically at 4, 10 or 25°C. On day 0, and after 2 and 4, 5 and 10, and 7 and 14 days of storage at 25, 10 or 4°C, respectively, microbial populations on meat samples were heat challenged (55°C) for up to 60 min by exposing a portion of homogenized sample to tempered broth. Bacteria were more heat resistant after storage at 25°C, compared to day 0. Initial populations of 7.30, 6.85 and 6.53 log CFU/cm^2 on C, HW- and LA-treated meat, respectively, were reduced by 3.37, 3.77 and 3.84 log CFU/cm^2, respectively, after 4 days storage at 25°C. *Salmonella* populations on LA-treated samples were more heat sensitive than C and HW-treated samples after storage at 10°C for 10 days; lower initial numbers of *Salmonella* on LA-decontaminated meat could account for this difference. Storage of meat at 4°C for 14 days did not alter the heat resistance of *Salmonella*. The results indicated that decontamination treatments and low storage temperatures, which control pathogen growth, may prevent increases in *Salmonella* heat resistance.

P146 Activated Lactoferrin Blocks *Escherichia coli* O157:H7 Interaction with Collagen Matrix and Beef Tissue

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Lactoferrin (LF) is a natural antimicrobial agent effective against a variety of bacteria, fungi, viruses, and parasites. LF could elicit stasis, cidal, opsonic and adhesion-blockade effects on susceptible organisms. Activated LF (ALF) is a defined formulation prepared by immobilizing the cationic N-lobe of LF molecule under controlled milieu conditions. This study is aimed to measure the ability of ALF to block *E. coli* O157:H7 interactions with collagen type-I (Cn-I) and beef tissue. Pre-treatment (1-h) of Cn-I surface with 2% ALF has blocked attachment of *E. coli* by 99.9% (~3-log) compared to the untreated Cn-I surface (control). Under similar conditions, 2% native LF elicited 40% (~1.6-log) adhesion-blockade effect. Detachment of *E. coli* from Cn-I surface by treatment with ALF and native LF at 2% concentrations showed a 99% (2-log) and 13% (~1.1-log) reduction respectively, when compared to controls. Efficacy of a simulated sanitizing spray system comprising lactic acid, hot water, cold-water rinse chambers to reduce *E. coli* on beef tissue was evaluated with or without 2% ALF rinse-step. Beef tissue was contaminated with *E. coli* O157:H7 (6 log CFU) labeled either with 3H-thymidine (by intrinsic DNA uptake) or green fluorescent protein (GFP) marker (by insertion of pGlo plasmid) to facilitate specific measurement of *E. coli* adherent to beef. Radioisotope counting of 3H-thymidine labeled *E. coli* indicated that the sanitizing system with and without ALF rinse showed 99.9% (~3-log) and 50% (~1.7-log) reductions, respectively. Also, the enumeration of GFP-producing *E. coli* on SMAC/arabinose-agar plates indicated similar results. These data suggest that ALF is a potent microbial blocking agent for beef safety.

P147 Electrostatic Spray Application of Activated Lactoferrin on Beef—Surface Distribution, Tissue Diffusion and Antimicrobial Activity

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Lactoferrin (LF) is a mammalian innate defense factor commonly found in milk, saliva, tears, and exocrine secretions that bathe the mucosal surface. This natural antimicrobial agent is also present in neutrophils that contribute to the endogenous pool of LF in animal tissue. Meat processing conditions wash off carcass surfaces and deplete the mucosal LF. We have developed an electrostatic spray (ESS) application for activated LF (ALF) to replenish this loss and to provide an exogenous antimicrobial barrier on beef surfaces. ALF (50 ml) was applied on a beef carcass by ESS in a 2-second burst and 7 tissue sites (in 6 replicates) were evaluated for spray coverage, concentration and antimicrobial activity. Samples were also tested for residual ALF after a 2% lactic acid wash followed by 10 to 12 h cooling in a chiller. Endogenous (tissue) levels of LF prior to ESS application were estimated at ~0.2 µg LF/gm of beef using an enzyme-linked immunosorbent assay (ELISA). Exogenous (ALF by ESS application) levels were measured at ~ 1.35 ± 0.1 µg ALF/sq cm of beef surface by use of a solid-phase immunoblot assay that utilized a Fluor-S Imaging system. No significant differences were observed in the exogenous ALF concentrations among the 7 sites of carcass samples ($P < 0.05$), suggesting a uniform surface distribution of ALF. Cross-section tissue blot analysis suggested that ALF diffused about 1 cm deep into the tissue in chilling conditions after 12 h. Challenge studies with fluorescent *E. coli* O157:H7 (containing pGlo-plasmid) indicated growth inhibition (stasis) of > 2 log bacteria/sq inch for 24 h with ALF-treated beef samples compared to control.

P148 Evaluation of Hot Water Immersion for Reduction of *Escherichia coli* O157:H7 on Beef Shanks

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At the request of a commercial processor, a study was conducted to determine the efficacy of hot water immersion for the reduction of *Escherichia coli* O157:H7 on contaminated beef shanks. Fresh beef shanks were inoculated with a cocktail mixture of streptomycin-resistant *E. coli* O157:H7 to facilitate recovery in the presence of background flora. Samples were submerged in water baths maintained at 87.8°C, 93.3°C or 98.9°C for 10, 20 or 30 s. Following treatment, 200 cm^2 of each shank surface was aseptically sampled using a hydrated sterile sponge. Numbers of *E. coli* O157:H7 were measured utilizing TSA with streptomycin. Inoculated shanks dipped in sterile deionized water prior to sampling were used as controls. For each time and temperature treatment combination, a minimum of a two-log reduction in viable *E. coli* O157:H7 was observed compared to the control. Generally, increased time and temperature resulted in increased lethality, with maximum reduction of approximately 3.5 log cycles observed for shanks

submerged for 30 s at 93.3°C and 98.9°C. Increased times and temperatures also resulted in increasingly undesirable surface color. These results illustrate that hot water immersion is an efficacious method to reduce *E. coli* O157:H7 concentrations on contaminated shank surfaces; however, final product quality is compromised with increasing immersion time and temperature.

P149 The Sources of *Escherichia coli* Contamination of Ground Beef in a Commercial Beef Processing Plant

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The objective of this study was to determine the sources of *E. coli* contamination of ground beef in a commercial beef processing plant by use of a Random Amplification of Polymorphic DNA (RAPD) technique. Samples for *E. coli* isolation were obtained from the hides (H), washed carcasses (WC), conveying equipment (CE), beef trimmings (BT) and ground beef (GB). During the summer of 2001 and winter of 2002, 150 and 100 *E. coli* isolates, respectively, were examined from each of those 5 sample sources. DNA patterns of *E. coli* isolates obtained with the RAPD method were compared to determine the genetically related or unique isolates among various sample sources. The data showed that the majority of the *E. coli* isolates were shared among the various sample sources; however, genetically diverse *E. coli* were also present. Most of the *E. coli* isolates recovered from the ground beef were similar to those recovered from WC, CE, BT and GB. Many unique *E. coli* strains found in the beef processing environment were distinct from those found on the hides of incoming animals. Randomly selected *E. coli* isolates were also analyzed by use of a pulsed-field gel electrophoresis (PFGE) technique to confirm the results obtained with the RAPD method, and the results were generally comparable. In conclusion, these data provide presumptive evidence that the *E. coli* found in ground beef can be derived from multiple sources in the commercial beef processing plant.

P150 A Comparative Heat Inactivation Study of Indigenous Microflora in Beef with That of *Listeria monocytogenes*, *Salmonella* Serotypes and *Escherichia coli* O157:H7

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Thermal inactivation of a mixture of five strains of *Listeria monocytogenes*, four strains of *Escherichia coli* O157:H7 and eight serotypes of *Salmonella* was compared to that of indigenous microflora in ground beef. Inoculated meat was packaged in bags that were completely immersed in a circulating water bath and held at 55, 57.5, and 60°C. The surviving cell population was enumerated by spiral plating heat-treated samples onto tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. The D-values, as determined by linear regression, in beef were 77.49, 21.9, and 10.66 min at 55, 57.5, and 60°C, respectively, for indigenous microflora. When either of the 3 pathogens were heated in beef, their calculated D-values were significantly lower ($P < 0.05$) than those of the indigenous microflora at all temperatures. Using a model for non-linear survival curves, the D-values of the major population (D1) at all temperatures for *L. monocytogenes* were significantly higher ($P < 0.05$) than those for *Salmonella* serotypes, *E. coli* O157:H7 or the indigenous microflora. However, higher recovery of a sub-population of the indigenous microflora in beef exposed to heating at 55, 57.5, or 60°C resulted in significantly higher D-values of the sub-population (D2) at all three temperatures, compared to those of the three pathogens at the same test temperatures. The results of this study will assist the retail food industry in designing acceptance limits on critical control points that ensure safety against *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* in cooked ground beef.

P151 Bactericidal Efficacy of GC-100X against Major Foodborne Pathogens and Detaching Effect of It against *Escherichia coli* O157:H7 on Beef

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The possibility of eliminating pathogenic microorganisms from meat has received considerable recent attention, and the use of organic acids was identified as one of the most practical options. However, organic acids have the potential to cause problems, including oxidation of myoglobin and corrosion of equipment, and to allow *Escherichia coli* O157:H7 to acquire acid resistance. Therefore, a new antimicrobial that does not have the demerits of organic acids is needed. GC-100X is a non-corrosive alkaline ionic water (pH 12) supplemented with xylitol, whose toxicological safety was verified through acute and sub-acute toxicological tests. An efficacy test of GC-100X was carried out against six major foodborne pathogens at three

different temperatures with or without organic load. Results revealed over 4 $\log_{10}$ (CFU/ml) reduction in all pathogens studied at 37°C for 3 h in the absence of the organic load. GC-100X solution two-fold diluted with distilled or standard hard water showed effective bactericidal activity, particularly against Gram-negative bacteria. Comparison of washing efficacy of GC-100X solution against *E. coli* O157:H7 on beef tissues with those of lactic acid and sodium lactate solutions was carried out through viable cell counts of *E. coli* O157:H7 and mesophilic bacteria at 0, 1, 3, 7, and 14 days. GC-100X stock solution was more effective than other tested solutions up to 7 days ($P < 0.05$). It did not compromise sensory qualities, including color and flavor, changes of which were observed in the lactic acid-treated group. These results indicate that GC-100X has good bactericidal and sanitizing activities, and is useful as a new meat-decontaminating agent.

P152 *Escherichia coli* O157:H7 Distribution in Beef Processed in a Table-top Bowl-cutter

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Beef processing equipment can be contaminated with pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. The bowl-cutter has wide application in particle-size reduction and blending of meat products. This study was undertaken to determine (1) the distribution patterns of *E. coli* O157:H7 in equipment components and ground beef produced with a table-top bowl-cutter, under different operational conditions; and (2) the likelihood that pathogen contamination can be transferred to subsequent batches, after a batch of beef contaminated with *E. coli* O157:H7 was processed in the same bowl-cutter. A beef trim inoculated with 2 log CFU of an *E. coli* O157:H7 strain resistant to rifampicin (*E. coli* O157:H7^{rif}) was fed into an uncontaminated beef trim batch under two different batch sizes, three processing times, and two feeding modes. There were no significant differences ($P \geq 0.05$) among all the treatments for the averages of the normalized counts of *E. coli* O157:H7^{rif} distributed in the ground beef. Regardless of the processing time and the method used to feed the beef trims into the bowl-cutter, the whole batch and at least the following subsequent batch became contaminated when previously contaminated beef was processed. Areas of the bowl-cutter that were most likely to be contaminated with *E. coli* O157:H7 were (1) the material left on the top of the comb/knife guard, when beef residues were tested, and (2) the knife, when the bowl-cutter surfaces were swabbed.

P153 Reduction of *Escherichia coli* O157:H7 in Refrigerated Ground Beef by *Lactobacillus* spp.

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A series of studies was conducted to determine if four strains of lactic acid bacteria (LAB) inhibited *E. coli* O157:H7 in laboratory media and in ground beef at 5°C. Frozen concentrated cultures of four *Lactobacillus* spp, NPC 747, NPC 750, D3, and L7, and a cocktail mixture of four strains of nalidixic acid resistant *E. coli* O157:H7 were used in this study. Nalidixic acid resistant strains of *E. coli* O157:H7 were used to facilitate recovery on non-selective media in the presence of the background flora. A 10^7 CFU/ml portion of individual isolates of the LAB were added to TSB broth containing 10^4 *E. coli* O157:H7/ml. Samples were stored at 5°C and the numbers of *E. coli* O157:H7 were determined on days 0, 5 and 10. After 5 days of storage, there were no significant reductions in the pathogen. However after 10 days of storage, all LAB reduced *E. coli* O157:H7 by an average of 3 log cycles. A second study was conducted in vacuum-packaged fresh ground beef. Samples were taken on days 0, 4, 8 and 12. The individual isolates resulted in an average reduction of 1.5 logs after 8 and 12 days of storage with little reduction after 4 days of storage. Following this study, a mixed concentrated culture was prepared from all four LAB and added to *E. coli* O157:H7 inoculated ground beef. After 7 days of storage, the mixed culture resulted in a 2 log reduction of *E. coli* O157:H7 compared to the control whereas after 14 days of storage, a 3 log reduction occurred. This study indicates that adding LAB to raw ground beef stored at refrigeration temperatures may be an important intervention to control *E. coli* O157:H7.

P154 Efficacy of Enrichment Broths Using BAX for Screening Recovery of Freeze-injured *Escherichia coli* O157:H7 in Inoculated Ground Beef

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Buffered peptone water (BPW), modified EC broth-novobiocin (mEC+n), and R & F enrichment broth (R&F-EB) were compared to detect freeze-injured *Escherichia coli* O157:H7 using the BAX®/PCR screening system. Twenty five-g ground beef samples inoculated with the freeze-injured *E. coli* O157:H7 were macerated in 225 ml of each broth, incubated under aeration at 41-42°C, and sampled after 6, 7, 8, and 24 h using BAX®/PCR screening.

Results were compared to the conventional procedure. For the aerated cultures, the broths were further incubated for 4 h during the BAX®/PCR protocol before confirmation. After 6 h, at an inoculation of 4.23 ± 1.00 total cells/25 g (74% freeze-injured), BAX®/PCR detected 72.7, 57.6, and 66% of the samples as presumptive positives from R&F-EB, BPW, and mEC+n broths, respectively, whereas, after 7 and 8 h, the recovery by BAX®/PCR exceeded 90% with R&F-EB at 100%. After 6 h, at an inoculation of 1.50 ± 0.56 total cells/25 g (80% freeze-injured), BAX®/PCR detected 47.6, 19.1, and 9.5% of the samples from R&F-EB, BPW, and mEC+n broths, respectively, and increased to 81.0, 61.9, and 52.4% after 7 h and 95.2, 61.9 and 71.4% after 8 h. After 24 h, 55 to 60% of the samples at both inoculum levels were detected by BAX®/PCR when conventional procedures were used, whereas, more than 95% of the samples were positive for BAX®/PCR with use of R&F-EB aerated at 41-42°C. In aerated R&F-EB and mEC+n broths, confirmations after 7 and 8 h closely correlated with the BAX®/PCR results. Thus, composition of the enrichment broth, aeration conditions, and incubation time and temperature (41–42°C), affect the efficacy of the BAX®/PCR to detect freeze-injured *E. coli* O157:H7.

P155 DSC Influence of Inoculum Level and Acidic Marination on Inactivation of *Escherichia coli* O157:H7 during Drying and Storage of Beef Jerky

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The objective of this study was to evaluate inactivation of *Escherichia coli* O157:H7 inoculated at Low (L) and High (H) levels (10^4 [L] and 10^7 [H] CFU/cm^2) on beef slices treated with two marinades, dried at 60°C for 10 h and stored at 25°C for 60 days. Initially, beef slices were inoculated with a five-strain composite and then treated with: i) nothing (C), ii) traditional marinade (TM), or iii) 5% acetic acid solution dip for 10 min followed by TM (AATM). After overnight storage (4°C), the slices were dried. Bacterial populations were determined during drying and storage, using tryptic soy agar with 0.1% sodium pyruvate (TSAP), sorbitol MaConkey agar (SMAC), and modified eosin methylene blue agar (MEMB). Total log CFU/g reduction of H in AATM samples from inoculation to 4 h of dehydration was greater (4.8–5.2) than the reduction of L (3.6–3.7); however, corresponding reductions by C and TM at H (2.5–2.9) were not different than at L (2.7–3.4) depending on agar medium. Under the conditions of this study, complete destruction (TSAP) of the L and H would occur in 8.8 and 16.1, 11.6 and 19.6, and 8.4 and 14 h of drying of samples of treatments C, TM and AATM, respectively. The data indicated that cell injury during drying was higher in C and TM than in AATM products, and drying also caused less injury at the L than H. The data suggested that the acidic treatment enhanced destruction of *E. coli* O157:H7 at both inoculation levels during drying and storage, while TM may have protected bacteria from inactivation.

P156 Nature of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Lactobacillus sakei* Inhibition by Eugenol, Cinnamaldehyde and Sodium Lactate

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We investigated the effects of natural antimicrobials, cinnamaldehyde, eugenol and sodium lactate, on foodborne pathogens *Escherichia coli* O157:H7 and *Listeria monocytogenes*, and the lactic acid bacterium *Lactobacillus sakei*. Log phase cells were grown in Trypto-Soy broth with 5 g/L yeast extract (20°C, pH 7.0), harvested at OD_{650} = 0.1 and diluted 1/1000 into fresh broth with the antimicrobial. Initial cell concentrations were 4.2 Log CFU/mL for *E. coli*; 4.7 for *L. monocytogenes*; 3.7 for *Lb. sakei*. The immediate response of the cells to the antimicrobials was determined by a spread plating of samples over a 1 h incubation. Cinnamaldehyde caused a reduction greater than 2 log CFU/mL at > 7 mM against E. coli and > 30 mM against *L. monocytogenes*. There was no effect against *Lb. sakei* at concentrations up to 0.5 M. Eugenol was more active with greater than 2 log CFU/mL reductions at concentrations of > 4 mM against *E. coli*, > 7 mM against *L. monocytogenes* and > 5 mM against *Lb. sakei*. Sodium lactate at pH 7.0 was tested at concentrations up to 1.3 M. Lactate had no lethal effect on *Lb. sakei* and *L. monocytogenes* but had a bacteriostatic effect over 6 h at > 0.5 M for *Lb. sakei* and > 0.3 M for *L. monocytogenes*. Lactate was bacteriostatic against *E. coli* over 6 hours at 0.3 M to 0.7 M and a 1 log CFU/mL drop was observed within 1 hour at 1.0 and 1.3 M. Bacteriocidal effects were confirmed by Iso-Grid plating of samples washed with 0.1% peptone.

P157 Occurrence of *Salmonella* on Poultry and Swine Farms

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The continued sustainability of poultry and swine industries has been questioned recently on the basis of their perceived contribution to the pollution of ground and surface waters and their potential role in spreading pathogens that might ultimately affect other animal or human popula-

tions. The objective of this study was to characterize the presence and populations of *Salmonella* contamination in broiler litter, fecal, litter drag swab and cloacal samples and swine feces under defined management systems and following passage through selected swine waste treatment technologies (ambient temperature anaerobic digester, solids separation/constructed wetlands system). For detection and enumeration of *Salmonella* species, 25-gram samples taken from multiple farms were first pre-enriched for 18 h at 37°C in filtered stomacher bags containing 50 ml of buffered peptone water and then selectively enriched in Rappaport-Vassiliadis broth for 18 to 24 h at 42°C. A 3 mm loopful of each serial dilution for each medium was subsequently streaked onto modified lysine iron agar and incubated at 37°C for 24 h. Suspect colonies were selected and confirmed by culturing on triple sugar iron agar slants and agglutination using poly-O antiserum. Broiler litter drag swabs and cloacal samples (n = 30) were *Salmonella* negative whereas over 33% and 50% of the litter (n = 30) and fecal (n = 30) samples, respectively, were *Salmonella* positive. The broiler litter and fecal sample mean and range of *Salmonella* populations were 3.5 log and 1.6 to 4.0 MPN/gram, respectively. No detectable *Salmonella* was found following passage through the swine waste management technologies (4 log reduction).

P158 Validation of Time and Temperature Values as Critical Limits for Ground Pork Processing and Storage

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In order to provide pork processors with valuable data to validate critical limits set for temperature during grinding, a study was conducted to determine *Salmonella* growth at various temperatures in raw ground products. Ground pork samples were inoculated with a cocktail mixture of streptomycin-resistant *Salmonella* to facilitate recovery in the presence of background flora. Samples were held at 4.4°C, 7.2°C, 10°C and room temperature (22.2°C–23.3°C) to mimic typical processing and holding temperatures observed in pork processing environments. *Salmonella* counts were measured, on TSA with streptomycin, at 2 h intervals for 12 hrs for samples held at room temperature. Samples held under refrigeration temperatures were sampled at 4, 8, 12, 24, 32, 48 and 72 h. Less than one log of *Salmonella* growth was observed at 48 h for samples held at 10°C. Samples held at 4.4°C and 7.2°C showed less than one log of *Salmonella* growth at 72 h. Significant ($P < .05$) increases in counts were observed at 32 and 72 h for samples held at 10.0°C and 7.2°C, respectively. These increases, while statistically significant, were less than 1/2 log. No significant increase in *Salmonella* counts was observed for samples held at 4.4°C. Samples held at room temperature showed no significant increase in *Salmonella* counts for the first 4 h; however, a significant increase was observed at 6 h. These results illustrate that meat processors can utilize a variety of time and temperature combinations as critical limits to minimize *Salmonella* growth during production and storage of ground pork.

P159 Validation of Time and Temperature Values as Critical Limits for Pork Fabrication and Storage

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In order to provide pork processors with valuable data to validate the critical limits set for temperature during fabrication, a study was conducted to determine the growth of *Salmonella* at various temperatures in raw, fabricated products. Growth of *Salmonella* was monitored in boneless pork chops held at various temperatures to determine growth patterns. Fresh boneless pork loins were sliced into approximately 2 cm thick portions. Boneless chops were inoculated with a cocktail mixture of streptomycin-resistant *Salmonella* to facilitate recovery in the presence of background flora. Samples were held at 4.4°C, 7.2°C, 10°C and room temperature (22.2°C – 23.3°C) to mimic typical processing and holding temperatures observed in pork processing environments. *Salmonella* counts were measured, on TSA with streptomycin, at 2 h intervals over 12 hrs for those held at room temperature. Samples held under refrigeration temperatures were sampled at 4, 8, 12, 24, 48 and 72 h. Less than one log of *Salmonella* growth was observed at 72 h for samples held at refrigeration temperatures. Significant ($P < 0.05$) *Salmonella* growth was observed at 48 and 72 h for samples held at 7.2°C and 10°C, respectively. No significant *Salmonella* growth was observed at 4.4°C. Samples held at room temperature exhibited less than one log of *Salmonella* growth at 10 h, with significant growth observed at 6 h. These results illustrate that meat processors can utilize a variety of time and temp-erature combinations as critical limits to minimize *Salmonella* growth during production and storage of raw, fabricated pork products.

P160 Changes in Swine and Cattle Production Practices Since the 1996 PR/HACCP Final Rule

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With the implementation of the 1996 Pathogen Reduction Hazard Analysis Critical Control Point (PR/HACCP) Systems Final Rule there has been

increased emphasis on on-farm food safety. These initiatives include the PR/HACCP rule itself plus increased producer education efforts; increased emphasis on on-farm food safety by producer organizations, FSIS, and state agricultural and animal health officials; and promotion of farm-to-table strategies by trade associations, industry, and academia. Swine and cattle producers' production practices may have been influenced by these collective efforts. To measure changes in swine and cattle production practices since the PR/HACCP rule, the authors conducted telephone interviews in fall 2000 and in spring 2002, with agricultural and extension officials and representatives from swine and cattle industries in nine states and nine national producer and veterinary organizations. Pre- and post-PR/HACCP data from the National Animal Health Monitoring System (NAHMS) were also analyzed. USDA's Food Safety and Inspection Service (FSIS) provided funding for this study. The findings suggest that PR/HACCP is pushing swine and cattle producers to examine their production practices and to think in terms of food safety. Through participation in quality assurance (QA) programs, producers have improved their drug administration, recordkeeping, and identification practices. Packers' requirements are driving QA program participation. Producers have improved their biosecurity practices because of concerns about bioterrorism and foreign animal disease. Producers' animal waste management and carcass disposal practices have stayed about the same. FSIS can use the study findings to guide future educational efforts with producers.

P161 Changes in the Identification and Control of Chemical Hazards since the 1996 PR/HACCP Rule

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This paper discusses the results of a survey of USDA FSIS plant personnel addressing the effects of the PR/HACCP farm-to-table initiatives on the identification and control of chemical hazards in establishments that slaughter high-risk market classes. Chemical hazards were defined as violative levels of animal drugs or pesticide residues. The purpose of the survey was to determine whether (and how) chemical hazards are being identified and controlled in establishments that slaughter high-risk market classes. A total of 34 veterinary medical officers (VMOs) were interviewed. These VMOs had an average of 10.5 years of service at their establishment(s). A total of 65 establishments that slaughter high-risk market classes were represented in the survey. Based on the survey responses and analysis of the data, the key findings of the survey are as follows. Since PR/HACCP implementation, establishments that slaughter high risk market classes are using a wider variety of approaches to identify and control chemical hazards. The majority of VMOs reported that while most of their establishments address chemical hazards in their hazard analysis, most do not have critical control points for chemical hazards. Most VMOs also reported that the majority of their establishments use one or more "best available" preventive practices to identify and control physical hazards. Other survey results are also discussed.

P162 Reduction of *Listeria monocytogenes* Populations during Exposure to a Simulated Gastric Fluid following Storage of Inoculated Frankfurters Formulated and Treated with Preservatives

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The effect of a simulated gastric fluid (GF; adjusted to pH 1.0 with HCl) on *Listeria monocytogenes* (LM), inoculated post-processing on pork frankfurters formulated with organic acid salts (sodium lactate/SL and sodium diacetate/SD) and treated by dipping in organic acid solutions (lactic acid/LA and acetic acid/AA), was evaluated during storage at 10°C for 40 d. Pork frankfurters containing antimicrobials either singly (1.8 % SL or 0.25% SD) or in combination (SL + 0.125% SD, SL + 0.25% SD) were inoculated (10^2 – 10^3 CFU/cm^2) with a 10-strain composite of LM and left untreated (ND) or dipped (2 min) in solutions (2.5%) of organic acids (LA or AA) before vacuum-packaging and storage. Survival of LM was determined after exposure (0, 20, 40 or 60 min) of frankfurters to the simulated GF at 0, 10, 20, 30 and 40 d. Survival of LM on frankfurters exposed to GF was higher as populations exposed to GF increased, which was directly related with treatments of reduced antimicrobial activity during frankfurter storage. Antimicrobials in the formulation did not appear to increase the GF resistance of LM survivors; however, dipping treatments, especially LA, appeared to increase resistance of LM to GF following growth during prolonged storage. Sodium diacetate (0.25%) included singly in the formulation increased the resistance of LM to GF on frankfurters dipped in LA. *Listeria monocytogenes* inoculated on freshly processed frankfurters may undergo rapid declines when exposed to GF, but after prolonged storage an acid-resistant sub-population may arise, provided the total population was > 4.0 log CFU/cm^2.

P163 Control of *Listeria monocytogenes* with Antimicrobials in the Formulation and by Dipping in Organic Acids of Post-processing Inoculated Pork Frankfurters Stored at 10°C in Vacuum Packages

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The study evaluated the antilisterial effect of sodium lactate (SL) and sodium diacetate (SD) in the formulation of frankfurters and in combination with dipping in solutions (2.5%) of lactic acid (LA) and acetic acid (AA) after processing. Pork frankfurters were prepared with 1.8% SL or 0.25% SD or combinations of 1.8% SL with 0.25 or 0.125% SD. After cooking and peeling they were inoculated (10^2–10^3 CFU/cm^2) with a 10-strain composite of *Listeria monocytogenes*, and left undipped or dipped (2 min) in organic acid solutions before vacuum-packaging and storage (10°C). Total mesophilic populations, *L. monocytogenes*, lactic acid bacteria (LAB), and yeasts/molds were determined during storage. In untreated, 1.8% SL and 0.25% SD samples, *L. monocytogenes* populations reached 6–8 log CFU/cm^2 in 12–20 days. The SL and SD combination treatments allowed increases of 0.1–1.7 log CFU/cm^2 in 28 to 40 days. Dipping in LA or AA reduced initial populations by 0.7 to 2.0 log CFU/cm^2, but during storage (12–20 days), growth in dipped samples without antimicrobials in the formulation reached 5.5–7.9 log CFU/cm^2. In samples containing single antimicrobials and dipped in organic acids, populations reached 1.3 to 2.3 CFU/cm^2 in 28 to 40 days. Bacteriocidal effects (reductions 0.6 to 1.0 log CFU/cm^2 in 28 to 40 days) were observed in frankfurters containing combinations of SL and SD and dipped in organic acids. No major growth of yeasts/molds was observed, while LAB proliferated in samples containing 0.25% SD, with or without dipping in LA. Combinations of antimicrobials in the formulation of frankfurters and dipping in organic acids can be more effective against *L. monocytogenes* when applied together.

P164 Recovery Rate of *Listeria monocytogenes* from Commercially-prepared Frankfurters during Extended Refrigerated Storage

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To assess the prevalence of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, about 33,000 packages, one pound each, were obtained by a third-party contractor from 12 volunteer commercial manufacturers over a two-year period. The 12 producers were comprised of 9 large and 3 small plants located in 8 USDA/FSIS districts in 10 states. Five days following manufacture, 500 packages were sampled using the USDA/ARS package rinse method. At regular intervals during subsequent storage at 4 and 10°C, an additional 200 packages were tested at each sampling point. From a statistical perspective, 9 producers did not have any packages/pounds which tested positive, whereas the pathogen was recovered at a rate of 1.5% (plant 367), 2.2% (plant 439), and 16% (plant 133) from product from the remaining three plants. In total, 532 of 32,800 (1.6%) packages/pounds from plants 133, 367, and 439 tested positive for the pathogen. The recovery rate did not change appreciably over time and there was no appreciable difference in recovery of the pathogen between storage at 4 or 10°C. Molecular subtyping of multiple isolates from each plant testing positive revealed that although isolates with a signature ribotype were recovered from each plant, it was also possible to recover isolates with more than one profile from a given plant. A single profile exhibiting a 1/2 a serotype, however, was displayed by 90% of 1102 isolates tested. These data establish the prevalence, types, and viability of *L. monocytogenes* associated with commercially-prepared frankfurters during extended refrigerated storage.

P165 Efficacy of Sodium Lactate and Sodium Diacetate Alone or Combined with Pediocin for Controlling *Listeria monocytogenes* in Ready-to-Eat Turkey Roll at 4°C and 10°C

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This study evaluated the efficacy of sodium lactate (SL) + sodium diacetate (SDA) alone or in combination with pediocin (ALTATM2341) for controlling *Listeria monocytogenes* in vacuum packaged ready-to-eat (RTE) turkey roll. SL (1,2, or 3%) + SDA (0.25%) or ALTATM2341 (6,000 AU), alone and in combination, were added to the formulation of turkey roll. The finished product was sliced and inoculated with a five strain mixture of *L. monocytogenes* to give 10^4 CFU/cm^2. Inoculated turkey roll without added SL, SDA, or ALTATM2341 served as controls. All samples were vacuum-packaged and held at 4°C (42 days) and 10°C (30 days). At set intervals during storage, populations of *L. monocytogenes* were determined by plating diluted samples onto Modified Oxford (MOX) agar and counting bacterial colonies on

MOX agar plates following 48 h of incubation at 35°C. Numbers of *Listeria monocytogenes* in controls increased rapidly and reached ~10^7 CFU/cm^2 on day 14 (4°C) and day 5 (10°C). All SL + SDA treatments alone or in combination with ALTATM 2341 inhibited inhibited growth of *L. monocytogenes* for 42 days at 4°C and 15 days at 10°C ($P < 0.05$). SL (2 or 3%) + SDA (0.25%) + ALTATM2341 completely inhibited growth of the pathogen for 42 days at 4°C. Results of this study demonstrate that SL + SDA in combination with ALTATM2341 can effectively inhibit the growth of *L. monocytogenes* in vacuum packaged RTE turkey roll particularly during storage at 4°C.

P166 A Predictive Model for Growth and Inactivation of *Listeria monocytogenes* in pH-Modified Chicken Salad during Cold Storage

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Ready-to-eat (RTE) foods such as prepared deli salads may become contaminated with *L. monocytogenes* due to contaminated ingredients and/or post-processing contamination. Combinations of preservation strategies can reduce the risk of contracting listeriosis from RTE foods. A conservative predictive model was developed to illustrate the growth and inactivation kinetics of *L. monocytogenes* subjected to varied pH (4.0–5.2) during storage at 5.0°, 7.2° and 21.1°C. Commercially produced pasteurized chicken salad was modified to pH 4.0, 4.6 and 5.2 by adding 2N acetic acid or 2M sodium acetate. 25g samples of the modified salad were individually inoculated to ~1 ¥ 10^6 cells/g with a mixture of *L. monocytogenes* (Scott A, LCDC 81-861, and F2365) and stored at the three temperatures for > 112 days. Samples were enumerated with the FDA modified Most-Probable-Number (MPN) procedure. Log MPN was plotted against time and results were compared to estimates from the USDA Pathogen Modelling Program (version 6.1). Inactivation was seen at all pH levels and all temperatures. At 21.1°C, a 6-log reduction was seen in 14 days at pH 4.0, 52 days at pH 4.6 and 38 days at pH 5.2. Inactivation began immediately at pH 4.0, and after a lag phase of 10–12 days, at pH 4.6 and 5.2. Inactivation rates were slower in cold storage, with decreases of 1.1 log (pH 5.2) and > 3 log (pH 4.0 and 4.6) after 119 days. The predictive model specifically addresses interactions between pH and temperature and can be used to develop formulation and storage guidelines for protein-based salads.

P167 Antibiotic, Biochemical, and Genotypic Characterization of Coagulase-positive *Staphylococcus aureus*

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The progressive emergence of antibiotic resistance in Staphylococci and its association with use and consumption of antibiotics constitute a major health concern and have been considered a global crisis. Twenty-five milk and miscellaneous samples were analyzed for *Staphylococcus aureus* using tryptone soy broth supplemented with 10% sodium chloride and sodium pyruvate as an enrichment broth and Baird-Parker agar supplemented with egg yolk-tellurite as a selective agar. Plates exhibiting dark color (presumptive positives) colonies were characterized biochemically with mannitol salt agar, catalase activity by slide spot test, deoxyribonuclease enzyme production with DNAase agar and *S. aureus* specific (clf A) gene by PCR assay. To determine the antimicrobial susceptibility profile, 8 different antimicrobial agents were tested by the disk diffusion method. Ten milk and miscellaneous samples (36%) were positive for *Staphylococcus.* A total of 280 isolates were obtained, of which 28 (10%) were positive for catalase, coagulase and deoxyribonuclease enzyme activity. Of the 28 isolates tested, 26 (93%) were positive for the clf A gene and these clf A positive isolates were tested for their antimicrobial susceptibility. Of the 26 beef strains tested, 27% of the *S. aureus* strains were sensitive, while 57% were resistant to antibiotics. This study indicates the use of antibiotics could possibly result in an increased trend of antibiotic resistant *Staphylococcus aureus* in milk products. This trend poses a threat in treating susceptible populations.

P168 Consumer Preferences for Labeling of Not-Ready-to-Eat Meat, Poultry, and Egg Products

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In response to the requirement to reassess their Hazard Analysis and Critical Control Point (HACCP) plans for ready-to-eat (RTE) meat, poultry, and processed egg products with respect to *Listeria monocytogenes* (Lm) control, some manufacturers have changed their products' processing categories. Consequently, the features on product labels that tell consumers whether a product is not-ready-to-eat (NRTE) or partially cooked, as opposed to RTE and

fully cooked, have become unclear. The authors conducted focus groups to assess consumers' perceptions and understanding of labeling terms and features that convey that products are NRTE and thus require cooking for safety before consumption. USDA's Food Safety and Inspection Service (FSIS) provided funding to conduct a total of 14 focus groups with consumers in seven locations. Participants expressed confusion about inconsistency in product labeling with regard to cooking for safety requirements. Many participants use information on product labels or packaging to determine whether products require cooking. To indicate that cooking for safety is required, participants preferred the use of logos (i.e., a frying pan icon with the phrase "Requires cooking"); standardized phrases, such as "Cook thoroughly"; and color-coding schemes with standardized phrases on NRTE product labeling. Many participants believed the government should mandate consistent labeling of NRTE products. In addition, some participants supported mandatory preparation instructions to help consumers safely prepare NRTE products. The study findings, in conjunction with other research, may help guide FSIS policy decisions regarding labeling of NRTE meat, poultry, and processed egg products.

P169 Changes in the Identification and Control of Physical Hazards since the 1996 PR/HACCP Rule

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This paper discusses the results of a survey of USDA FSIS Circuit Supervisors (CSs) on the effects of the PR/HACCP farm-to-table initiatives on the identification and control of physical hazards. Physical hazards (foreign objects) were specifically defined as bone, glass, metal, and plastic in the survey. The purpose of the survey was to determine whether (and how) physical hazards are being identified and controlled in federally inspected establishments. Thirty-four CSs were randomly selected, two per FSIS district, and interviewed. These CSs had an average of 10 years of service with FSIS. The circuits participating in the survey represented 1,024 federally inspected meat and poultry establishments. Based on the survey responses and analysis of the data, the key findings of the survey are as follows. Since PR/HACCP implementation, meat and poultry establishments are overall using more methods and approaches for identifying and controlling physical hazards. Audits of suppliers in particular have increased since PR/HACCP implementation. Most establishments address physical hazards in their hazard analysis and often identify metal detection as a critical control point (CCP) in their HACCP plan. However, metal is the only physical hazard for which a CCP is frequently identified. Other survey results are also discussed.

P170 Cell Surface Attachment of *Listeria monocytogenes* on Ready-to-Eat Meats

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Cell surface attachment is characterized by net negative charge and hydrophobicity. Electrostatic interaction chromatography (ESIC) and cationized ferritin (CF) are methods chosen to study net negative charge distribution on the bacterial cell surface. Hydrophobic interaction chromatography (HIC) and contact angle measurement (CAM) were used to examine the cell surface hydrophobicity. The ratio of strongly attached (sessile) *L. monocytogenes* cells compared to total (sessile and planktonic) attached cells on RTE meats was also determined. Five individual strains of *Listeria monocytogenes* and a mixed cocktail of all five were studied on frankfurters, ham, bologna, and roast beef. No evidence of a difference ($P > 0.05$) was observed in cell surface charge and cell surface hydrophobicity among strains. Approximately 84 to 87% *L. monocytogenes* was found to attach strongly to RTE meats in 5 min. No evidence of a difference ($P > 0.05$) was found among strains and among meats. Micrographs from scanning electron microscope (SEM) showed no visual difference among the strains but showed a difference in age of cells (mixed culture) in terms of surface negative charge distribution. More negative sites were observed at day 0 and day 7 and many fewer at day 3, indicating possibly a change in cell surface properties. Since no difference in strains was established, the CAM was carried out using the five-strain mixed culture. Inoculated (10^8 CFU/ml) and non-inoculated RTE meats with *L. monocytogenes* showed evidence of a significant difference ($P < 0.05$).

P171 Combinations of Nisin and Gamma Irradiation for Effective Control of *Listeria monocytogenes* on Meat

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Preservation of meat by ionizing radiation may lead to undesirable sensory changes. Using low doses of irradiation in combination with complementary treatments may minimize these undesirable changes. Therefore, inactivation of *Listeria monocytogenes* on meat by nisin and low doses of

gamma irradiation was studied. *Listeria monocytogenes*, in buffered saline or on meat cubes, was treated with nisin (4×10^4 IU/mL) for 30 min, irradiation (0.25–1.5 kGy) or their combinations. Treated meat cubes were analyzed immediately for *L. monocytogenes* or stored at 4°C for 24 or 72 h before analysis. Nisin or gamma irradiation alone inactivated *L. monocytogenes* in buffer and on meat cubes, but the combinations of these agents were even more effective. Nisin caused 1.8 and 1.5 log units reduction of *L. monocytogenes* in buffer and meat, respectively. The D-values of *L. monocytogenes* in buffer and on meat, due to treatment with irradiation alone, were 0.16 and 0.47–0.49 kGy, respectively. D-values of *L. monocytogenes* due to combination treatments were 0.13 in buffer and 0.41–0.47 on meat. Different inactivation rates were observed when the order of applying nisin and gamma irradiation was changed. When inoculated meat was treated with nisin followed by 1.5 kGy gamma irradiation, the count of *L. monocytogenes* was $<10^2$/g after 72 h refrigerated storage. In conclusion, treatment of *L. monocytogenes* with combinations of nisin and low doses of gamma irradiation is effective in controlling *L. monocytogenes* on meat.

P172 Safe Prediction Zone, a New Method for Validation of Predictive Models

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Predictions of broth models for lag time (LT) and specific growth rate (SGR) of *Salmonella* Typhimurium were compared with observed LT and SGR on sterile cooked chicken breast and thigh burgers. The experimental conditions and modeling methods were the same for the broth models and sterile cooked chicken burgers so as not to confound the comparisons. Relative error (RE) plots of broth model predictions were evaluated for prediction bias and accuracy, using a safe prediction zone (SPZ) that ranged from RE of -0.3 to 0.15, where positive RE represented fail-dangerous predictions and negative RE represented fail-safe predictions. Relative errors outside the SPZ were considered overly fail-dangerous or overly fail-safe. The fraction of RE that were within the SPZ (RESPZ) was calculated and a minimum RESPZ of 0.85 was established and used as the sole criterion for model validation. For LT, 16 of 24 RE were within the SPZ, for a RESPZ of 0.64, whereas for SGR, 21 of 24 RE were within the SPZ, for a RESPZ of 0.88. Thus, the broth model for LT was not validated (i.e., RESPZ < 0.85), whereas the broth model for SGR was validated. It is of note that the broth model for LT provided overly fail-dangerous predictions of *S.* Typhimurium growth on sterile cooked chicken burgers at short LT (< 4 h).

P173 Development and Evaluation of a Mathematical Model for the Effect of Temperature, pH, NaCl and Sodium Lactate on the Surface Growth Limits of *Listeria monocytogenes*

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Modeling microbial growth/no growth boundaries describes the range where multiple environmental factors begin to interactively prevent growth. The objective was to develop and evaluate a model for the growth/no growth interface of *Listeria monocytogenes* as a function of temperature, pH, NaCl and sodium lactate concentration. Growth initiation of a *L. monocytogenes* 5-strain composite on tryptic soy agar (TSA) was studied in 875 combinations of temperature (4–30°C), pH (4.51–6.44), NaCl (0.5–12.5%) and sodium lactate (0–10%). Growth/no growth was determined periodically for 60 days by visual observation of colony formation. Absence of growth was confirmed by re-plating on TSA and comparing resulting populations with the inoculum size (Student's t-test). Data were modeled using logistic regression. The performance of the model was evaluated by comparing predicted growth limits with observed responses of *L. monocytogenes* in meat products of different sodium lactate concentrations. The model fitted the data with a concordance of 99.9%, and allows for the prediction of the minimum concentration of sodium lactate preventing growth of *L. monocytogenes* at different temperature, pH and NaCl levels. At 25°C and pH 5.5 the predicted sodium lactate limits for growth were 4.65% and 3.11% for 2.5% NaCl and 4.5% NaCl, respectively. Comparison of the model's prediction with observed growth of *L. monocytogenes* in meat products showed a satisfactory agreement between predicted and observed responses. In 18 cases where growth was observed in meat products the predicted probabilities were higher than 0.99. In one case where the pathogen did not grow in the product the model predicted a probability of 0.608.

P174 Enumeration of *Salmonella* with the Polymerase Chain Reaction BAX System and Simulation Modeling

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Concepts of microbial growth kinetics, polymerase chain reaction (PCR) detection of pathogens and simulation modeling were combined to develop a predictive model for initial contamination of chicken with *Salmonella*. Kinetic data from challenge studies with a single strain of *Salmonella*

Typhimurium and chicken homogenates (25 g:225 ml) were used to build the model. Chicken samples were inoculated with 0 to 6 log CFU of *S.* Typhimurium followed by incubation at 37°C for 24 h. Sub-samples were collected during incubation and tested for *S.* Typhimurium by use of the PCR BAX system with a sensitivity of 4 log CFU/ml. A detection time score (DTS) based on the widths of the PCR gel bands for the sub-samples was obtained for each inoculum level. Standard curves relating PCR DTS to inoculum level were developed using a two-phase linear model. Uncertainty of the best-fit values for standard curve parameters was modeled using probability distributions and Monte Carlo simulation. A simulation model was designed to predict the distribution of *Salmonella* contamination among any size sample of chicken that was a multiple of 25, the grams of chicken used to build the model. Type of sterile chicken meat did not affect the shape of the standard curve, whereas microbial competition did. Presence of competing microorganisms suppressed growth, and thus PCR DTS, at low but not at high inoculum levels. Simulation results indicated that distribution of *Salmonella* contamination increases in a non-linear manner, as a function of sample size, and thus linear extrapolation of enumeration results is not appropriate.

P175 Modeling the Effects of Food Handling Practices on the Incidence of Foodborne Illness

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Each year millions of cases of foodborne illness occur in the United States. Preceding most cases of foodborne illness is contamination of food by pathogens and failure of retail food establishments or households to destroy or sufficiently control pathogens before consumption. This paper presents results of a research project sponsored by the Food and Drug Administration to develop a quantitative model for estimating the effects of food handling practices and other contributing factors on the incidence of foodborne illness. Working collaboratively with FDA, RTI International developed the Food Handling Practices Model (FHPM) to provide a quantitative method for estimating the benefits of changes in food handling practices. The model incorporates the inherent uncertainty of key relationships involved by using stochastic simulation methods. Because unsafe food handling practices and other contributing factors occur in households, retail food establishments, and institutional establishments, the FHPM explicitly models all three venues. The FHPM operates by use of Microsoft Excel™ combined with Microsoft Access™ and the add-in application software @Risk™. A user-friendly interface allows users who are unfamiliar with Excel, Access, or @Risk to use the model effectively and efficiently. Combining data from experimental research, prevalence studies, outbreak studies, and environmental health surveys, the FHPM also provides a new approach for estimating annual cases of foodborne illness that may be attributed to the retail channel versus the household channel. We anticipate that the new model will be useful for guiding additional primary research to measure the incidence of contributing factors and the antecedents to foodborne illness.

P176 A Quantitative Risk Assessment Model for *Salmonella* and Whole Chickens at Retail

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A quantitative risk assessment model (QRAM) was developed for predicting the rate of salmonellosis from whole chickens at retail. The QRAM was created in an Excel spreadsheet and was simulated using @Risk. The retail-to-table pathway was modeled as a series of pathogen events that included initial contamination at retail, growth during consumer transport, thermal inactivation during cooking, cross-contamination during serving and dose-response after consumption. Data from the scientific literature and data from consumer surveys of food handling practices as well as predictive models for growth and thermal inactivation of *Salmonella* on chicken were used to establish input settings. Simulation results indicated that the most highly contaminated chickens at retail did not result in the greatest exposure to *Salmonella.* Rather, by random chance, whole chickens with lower levels of *Salmonella* at retail resulted in greater consumer exposure when they were undercooked and mishandled during serving. *Salmonella* growth on raw chicken during consumer transport was the only pathogen event that did not impact the rate of salmonellosis. For the scenario simulated, the QRAM predicted a rate of 0.44 cases of salmonellosis per 100,000 consumers, which was consistent with recent epidemiological data that indicates a rate of 0.66 to 0.88 cases of salmonellosis per 100,000 consumers of chicken. Although results of the QRAM were in agreement with the epidemiological data, many assumptions were made because of data gaps and thus, further refinement of the model is needed before its predictions can be considered reliable.

P177 Quantitative Microbial Risk Assessment of the Sprout Production Process

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Consumption of raw sprouts has been of growing concern over the past decade as they have been linked with a number of foodborne disease

outbreaks. A quantitative microbial risk assessment (QMRA) was created to determine the most effective points for pathogen control in the sprout production process. Published literature was reviewed, applicable data compiled, and histograms created in Excel. Appropriate statistical distributions were determined using Bestfit software and were used to create a quantitative risk assessment in Analytica. Factors modeled in the QMRA included initial pathogen concentration, method of seed disinfection, and seed sampling prior to sprouting. Sprouting conditions such as rinse frequency, temperature of water and chlorination of water were also analyzed. Finally, methods of sprout disinfection, sprout sampling (none, sampling sprouts, and sampling rinse water), and the effect of different storage conditions were examined. No type of seed disinfection completely eliminated pathogens. Sprout sampling and rinse water sampling at the end of the sprouting process were more effective in pathogen detection than seed sampling. Increased rinse frequency and decreased temperature during sprouting retarded pathogen growth. Storage conditions after sprouting had a minimal effect on contamination. The results of this QMRA suggest that the incidence of sprout-related outbreaks could be reduced by using a combination of seed disinfection, proper sprouting conditions, and sprout or rinse water sampling. This QMRA can also be used to guide further research and compare the effectiveness of different risk reduction strategies.

P178 Generalized Extreme Value Distributions for Risk Assessment: A Monte Carlo Study

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The necessity of Quantitative Risk Assessment (QRA) in food safety risk characterization is widely recognized. Risk Assessment, using statistical techniques, requires the specification of probability distributions for the variables of interest, and often the Triangular and Beta distributions are used. When the extremes of a variable are of interest then the Extreme Value distributions, which are probability distributions devised to study the behaviour of sequences of extremes, can be used. To be successfully implemented, Maximum Likelihood estimation needs sample sizes "large enough", but in practical applications this condition is not often fulfilled. In this paper we present the results of a Monte Carlo simulation study of the small sample properties of the Maximum Likelihood estimators of the Generalised Extreme Value (GEV) distribution. The simulation was implemented in the R language. For each of four different sample sizes, n=10, 15, 20 and 25, M=20000 samples from the GEV distribution were simulated. From these simulations the Coverage and Bias were estimated for each of the parameters defining the GEV distribution. The results obtained suggest that, in terms of Bias and Coverage, for small sample sizes, say $n < 20$, the Maximum Likelihood Estimators perform satisfactorily and estimation of the GEV using small sample sizes appears as a feasible alternative.

P179 Risk Assessment and Risk Communication for *Listeria monocytogenes* in Ready-to-Eat Foods with a Focus on Food Handling at Home

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Estimates from surveillance data indicate that at least 50% of foodborne diseases is caused by poor food handling at home. However, consumers believe that responsibility for food safety problems lies primarily with food manufacturers, retailers, institutions, or possibly food preparation by restaurants or other individuals. Preliminary data revealed that many consumers lack the motivation to change their food preparation behaviors because of overconfidence in their own food handling practices. This paper describes a risk assessment model that incorporates new data on consumers' attitudes and food-handling behaviors based on personal interviews, focus groups and a survey of 1000 households. We defined "appropriate" and "inappropriate" practices for preparing ready-to-eat (RTE) foods at home and assessed the associated risk from *Listeria monocytogenes*. The model expands the FDA risk assessment model for *Listeria monocytogenes* in RTE foods, focusing on three popular foods categorized as high risk in the FDA model: deli meat, deli salad, and frankfurters. Cross-contamination during shopping, storage and food preparation, as well as temperature and time for transportation, refrigeration, and handling of left-overs, were modeled. Risks were assessed for perinatal, elderly and intermediate-age subpopulations. The most critical practices for different food categories were identified by sensitivity analysis. The results of the risk assessment will be used for designing risk communication messages for consumers. A consumer self-evaluation of his/her own food handling behaviors, followed by communication of the relative risk associated with those behaviors, may promote consumer motivation to acquire more food safety knowledge and to change behavior.

P180 *Lactobacillus casei* Viability after Impregnation into Apple Porous Structure

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Consumer demand for functional products has generated an important change; probiotic bacteria have been included into several foods, especially dairy products. Incorporation of probiotics into solid foods porous structure can be done through vacuum impregnation (VI) but the process needs to be evaluated in terms of microbial viability. The application VI process at 25°C to incorporate *Lactobacillus casei* into the porous structure of apple pieces of different varieties was evaluated determining probiotic viability and product acceptability. Apple (Golden delicious, Starking and Granny Smith) effective porosity was determined employing VI in isotonic sucrose solutions, obtaining values around 25%. Impregnation media was prepared by centrifuging and washing *L. casei* cells from an 18 h culture broth, and suspending them in apple juice reaching 10^9 CFU/mL. Fruit cubes (1.1 cm) were submitted to VI processes (50 mbar) in apple juice with *L. casei*. Fruit-juice ratio was kept constant (1–3), and impregnation and relaxation times were fixed to 10 min each. *L. casei* viability was evaluated by spread plating into MRS agar and determining CFU/g after 24 h incubation at 35°C under anaerobic conditions. ANOVA comparing *L. casei* incorporation into different apple varieties demonstrate a non-significant difference ($P > 0.05$) in final numbers, reaching populations around 10^8 CFU/g. For every apple variety, triangle comparison sensory tests performed between apples with or without *L. casei* incorporation results in a non-detectable difference ($P > 0.05$). When the acceptability (9 point hedonic scale) of *L. casei* impregnated apples was evaluated, no significant difference ($P > 0.05$) was observed; scores obtained were over 7.0.

P181 Inhibitory Activity of *Bifidobacterium longum* HY8001 against *Salmonella enterica* serovar Typhimurium DT104

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Currently, there is a growing interest in probiotics with increasing occurrence of antibiotic resistant bacteria due to overuse of antibiotics. In this study, we focused on analysis of inhibitory effects of *Bifidobacterium longum* HY8001, Korean isolate, against *Salmonella enterica* serovar Typhimurium DT104 infection in vitro and in vivo. Antimicrobial activity was determined by the agar dilution method, using the culture supernatant of *B. longum* HY8001 with pH 7.2 adjusted to confirm that it secretes antimicrobial substances other than lactic acid, and that it had antimicrobial activity against *S.* Typhimurium DT104. Adhesion and colonization property of *B. longum* HY8001 and its inhibitory effects against *S.* Typhimurium DT104 were also determined in vitro using Caco-2 cell. *B. longum* HY8001 and its culture supernatant itself strongly inhibited the adhesion and invasion of S. Typhimurium DT104 into Caco-2 cell at pH 7.2, as well as having adhesive property. We also investigated its inhibitory effect using a mouse ligated intestinal loop model. Intestines of mice treated with *B. longum* HY8001 and its culture supernatant were protected from most of damage and the villi also had essentially normal architecture, in contrast to intestines without *B. longum* HY8001 treatment, which had extensive of morphologic damage. These results suggest that *B. longum* HY8001, Korean isolate, could protect against infection by multi-drug resistant *S.* Typhimurium DT104 by producing antimicrobial substances and preventing *S.* Typhimurium DT104 invasion of intestinal epithelium.

P182 Characterization of *Listeria innocua* Biofilm Formation Using Tn917 Transposon Mutagenesis

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Listeria sp. can grow on surfaces and be a member of multi-species biofilms within food processing plants, rendering them more resistant to sanitizers and other antimicrobial agents. Little is known of the cellular physiological processes involved in *Listeria* sp. growth on surfaces. The purpose of this study was to generate random transposon mutants in order to identify genetic determinants contributing to biofilm formation. *Listeria innocua* ATCC 51742 serotype 4b was selected for this study since it produces high levels of biofilm in a laboratory assay. Transposon mutagenesis was performed using plasmid pTLV3 (Tn917) with a temperature sensitive origin of replication. Mutants were selected by growth in the presence of erythromycin and lincomysin at 41°C. A library, consisting of 2500 mutants, was further screened by use of a PVC microtiter plate biofilm assay at 32°C at low nutrient levels. Reduced surface growth (RSG) mutants were defined as having a mean stained biofilm at 2 std dev below the mean of the wild type strain. A total of 35 RSG mutants were isolated and further characterized by testing cellular motility and initial adhesion phenotypes. Phenotype screening showed RSG's exhibited varied profiles, suggesting a number of genes are involved in biofilm formation of *L. innocua*. Genetic

determinants were cloned into *E. coli* HB101 ATCC 33694, utilizing a ColE1 origin of replication and a kanamycin (neo) selection within Tn917, and sequenced. Knowledge of essential biofilm formation genes may eventually lead to unique strategies for biofilm prevention and removal in food processing environments.

P183 Characterization of a Swarming Phenotype of *Listeria innocua* on Semi-solid Surfaces

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Biofilms are microbial communities existing in abiotic and sometimes biotic surfaces. Once established, biofilms appear to have a greater resistance to antimicrobial agents compared to planktonic organisms, and this has also been observed with *Listeria* sp. biofilms. *Pseudomonas aeruginosa* biofilm formation has been related to a swarming phenotype. Swarming in *Listeria innocua* (defined as an organized movement of bacteria over a solid surface) was induced on semisolid surfaces in MWA (modified Welshimer's broth with 0.4% agarose). This phenotype was studied under different temperature conditions (4, 20, 32, 37, and 44°C), with glucose as a carbohydrate source, and under conditions of different carbohydrate sources (glucose, fructose, cellobiose, and maltose) at 32°C. Greatest swarming was observed at 32°C with glucose as the carbon source. Maltose, cellobiose, and fructose induced swarming but to a very smaller degree. A mutant in flagellin (flaA) was constructed by use of a temperature sensitive insertion vector. Motility negative phenotype was screened using motility test agar and confirmed using electron microscopy. Experiments were performed to determine whether flagella are needed in swarming and biofilm formation by *L. innocua*. To our knowledge, this is the first report of swarming motility in *L. innocua* and this phenotype may be important in the growth and persistence of this organism on food processing surfaces.

P184 Comparing the Efficacy between Single and Double Pulse Pressure-assisted Thermal Processing on Inactivation of *Bacillus stearothermophilus* ATCC 10149 Spores

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Pressure assisted thermal processing involves the simultaneous application of elevated pressures (up to 900 MPa) and temperatures (up to 120°C) with shorter processing times. It provides an attractive alternative to traditional thermal based methods for processing low-acid foods. *Bacillus stearothermophilus* has been used as a model surrogate organism for validation of low-acid thermal processes. The microbial efficacy between single and double HPP process was evaluated using *B. stearothermophilus* ATCC 10149 spores as a test microorganism. The spores (10 ml, 10^6 CFU/mL) were suspended in de-ionized water and placed in sterile high barrier nylon pouches. A pilot scale high-pressure food processor (Quintus Model QFP-6, Flow Autoclave Systems, Columbus, OH) equipped with an internal heater was used to process the test pouches. Samples were preheated to the target initial temperature and treated at 90 and 110°C, pressures from 400 to 700 MPa and holding time of 0.01 and 120 s, conditions for single and double pulse HPP. Samples were serially diluted, spread-plated on TSA and incubated at 55°C for 36–48 h. Results suggest that the microbial effectiveness of single and double pulse primarily depended upon the pressure-temperature treatment conditions. At lower process temperatures (90°C), for the range of pressures tested, double pulse HPP showed higher inactivation of *Bacillus* spores than the single pulse HPP. As the process temperature increased to 110°C, the difference between single and double pulse was minimal for all the pressures tested when the spores were treated for 2 min.

P185 DSC Starvation-induced Cross-protection of *Escherichia coli* O157:H7 against Electron-beam Irradiation in 0.85% Saline and in Apple Juice

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This study evaluated the effect of starvation on the resistance of starved *Escherichia coli* O157:H7 to electron beam irradiation in 0.85% (w/v) saline and in apple juice. Exponential-phase cells of *E. coli* O157:H7, grown at 35°C in tryptic soy broth (TSB), were sedimented by centrifugation (10,000 x g, 10 min, 4°C), washed, then starved in 0.85% saline (25°C) for 10 days. Exponential- or stationary-phase cells grown in TSB at 35°C served as controls. Samples of 0.85% saline or pasteurized apple juice, inoculated with control cells or cells starved for 8 days, were exposed to electron beam irradiation at doses ranging from 0.0 to 0.7 kGy. *E. coli* survivors were enumerated by plating diluted samples on tryptic soy agar (TSA) and counting bacterial colonies on TSA plates after incubation (35°C) for 24 h. Starved cells consistently exhibited higher irradiation D10 values than controls ($P < 0.05$). D10 values for control and starved *E. coli* O157:H7 in 0.85% saline were 0.11 and 0.26 kGy, respectively; D10 values in apple juice were 0.16, 0.19, and 0.33 kGy for exponential, stationary, and starved cells, respectively. Irradiation (0.7kGy) of *E. coli* O157:H7 in apple juice reduced numbers of exponential- and

stationary-phase cells by ~ 4.32 and 3.74 log, respectively, whereas starved cells were reduced by only 2.20 log. The results of this study indicate that starvation-induced stress cross-protects *E. coli* O157:H7 from the irradiation treatment and it should be considered as an important factor when determining irradiation D10 values for this pathogen.

P186 Inhibition of Selected Fungi by Psoralen - Long Wave Ultraviolet Light

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Psoralen is a furocoumarin naturally synthesized in some plants; this compound is activated as an antimicrobial system by exposure to long wave ultraviolet light (UVA). The inhibitory effects of psoralen concentration (10, 30 or 50 ppm) exposed or not to UVA (365 nm) at selected distances (5, 10 or 15 cm) for 1, 3 or 5 min on a cocktail of *Aspergillus flavus*, *A. niger*, *Zygosaccharomyces bailii* and *Z. rouxii* in potato dextrose agar (PDA) adjusted to pH 3.5 and a_w, 0.97 were evaluated. Triplicate plates of every combination were inoculated with a cocktail of mold spores and yeast cells suspension, exposed or not to UVA, incubated for 30 days (25°C), and periodically observed for growth. If growth was observed the response was 1, if not it was registered as 0. Backward stepwise logistic regression was used to develop a simplified model able to predict fungal probability of growth. The increase in psoralen concentration, exposure to UVA, and shorter distances inhibited microbial growth. Microbial growth was observed in 19 cases from a total of 51 observations. The model shows that exposure distance reduction increased the number of combinations of psoralen concentration and exposure time with probabilities to inhibit growth higher than 0.95. The obtained model predicts the probability of growth under a set of conditions and can be used to calculate critical values of psoralen concentration, exposure times or distance needed to inhibit fungal growth for different probabilities. The results obtained suggest that psoralen – UVA could be used as an antimicrobial system to prevent fungal spoilage.

P187 Inhibitory Activity of *Lactobacillus reuteri* SD 2112 against Vero Cytotoxin of *Escherichia coli* O157:H7

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Escherichia coli O157:H7 is an emerging foodborne pathogen worldwide and its cytotoxin (VT) is associated with haemorrhagic colitis (HE) and haemolytic uremic syndrome (HUS) in humans. Attachment of VT to its receptor, globotriaosylceramide (Gb_3) at gut epithelium is the primary step and, subsequently, VTs induce cell death by inhibition of protein synthesis. Proinflammatory cytokines, such as TNF-a and IL-b, upregulate Gb_3 expression, increase sensitivity to VTs, and enhance VT action in promoting disease. Our previous study showed that *Lactobacillus reuteri* SD 2112 had antimicrobial activity against *E. coli* O157:H7. Therefore, this study focused on unraveling inhibitory effects of *L. reuteri* SD 2112 against VTs. The culture supernatant of *L. reuteri* prevented the cytopathic effect (CPE) by VT in Vero cells and the culture supernatant, treated with a reuterin production inhibitor still prevented the CPE by VT. In the competitive ELISA, we found that there were substances in the culture supernatant of *L. reuteri* which interrupted conjunction of VT and Gb_3. Mice were challenged with *E. coli* O157:H7 after treatment with *L. reuteri* culture supernatant. IL-1b levels in sera were decreased and expression of Gb_3 in renal tubular epithelial cells was reduced in mice treated with *L. reuteri* culture supernatant. To sum up these results, it is considered that *L. reuteri* SD 2112 showed VT neutralizing capability primarily through inhibition of conjunction of VT and Gb_3, and decreased IL-1b production and Gb_3 expression also contributed to the effect.

P188 Inhibition of *Aspergillus flavus* by Sourdough Lactic Acid Bacteria

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Lactic acid bacteria (LAB), because of their antibacterial and antifungal activity, have a great potential for industrial use as biopreservative agents. The antifungal activity of 8 strains of LAB, isolated from 2 different sourdough bread cultures, was examined. *Aspergillus flavus* NRRL 1290 was used as indicator strain according to its importance as possible aflatoxin producer. To determine the inhibitory effect of sourdough LAB, a dual culture agar plate assay was used. Commercial de Man-Rogosa-Sharpe (MRS) agar and modified MRS (mMRS) agar (without sodium acetate) was used for cultivation of LAB. Each strain of LAB (1% of grown culture in MRS or mMRS broth, 37°C, 18 h) was inoculated into 15 mL of MRS or mMRS agar. After solidification, plates were overlaid with soft (0.75 % of agar) Potato-Dextrose agar (PDA). Mold spores

(103) were spotted onto the PDA surface in the center of the plate. Colony diameter of the growing mold cultures was measured every day for three weeks. Mold growth and sporulation was completely inhibited by 6 out of 8 strains of LAB grown on commercial MRS agar. O2 and O11 cultures isolated from Original sourdough bread culture and grown on commercial MRS agar delayed mycelial growth and spore production by 7 and 6 days, respectively. All strains of LAB grown on mMRS delayed mold growth and sporulation by 4 to 6 days. There were no significant differences among the inhibitory effects of the individual strains of LAB.

P189 Effect of Combined Protamine and Heat Treatments on Survival and Release of Surface Proteins of Wild-type and Protamine Resistant *Listeria monocytogenes* Scott A

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Protamine is a cationic antilisterial peptide (CAP) from fish sperm. However, *Listeria monocytogenes* frequently develops resistance towards CAPs, potentially limiting their use in food. The objective of this study was to investigate the effect of heat and protamine on survival and expression of surface proteins of *L. monocytogenes* Scott A (Lm) and its protamine resistant mutant (PrtR). Lm and PrtR were exposed to heat (50, 55 or 60°C) alone or with sub-lethal concentrations of protamine (250 ppm) in tryptic soy broth and enumerated on tryptic soy agar with 1% pyruvic acid. Released surface protein patterns were analysed by two-dimensional gel electrophoresis after heat shock (50°C, 30 min) with or without protamine (250 ppm) and compared to controls (37°C). At 50°C, D-values were 70 min for both strains. D-values at 55 and 60°C of 13.4 min and 1.0 min for PrtR were significantly lower than the D-values of 28.4 min and 1.3 min, respectively, for Lm. Protamine reduced D-values for both strains at all temperatures. Heat shock induced a common protein in both strains while suppressing more than 50% of proteins in PrtR and up-regulating about six proteins in Lm. PrtR always expressed fewer proteins than Lm. Protamine reduced the number of proteins expressed by both strains after heat shock and decreased expression of surface proteins correlated with decreasing D-values. In conclusion, PrtR was less resistant than Lm to heat treatments with or without protamine, indicating such mutants may not be a concern when using combined treatments.

P190 Inactivation of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in Frozen Ground Beef Patties by Electron Beam Irradiation

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The inactivation of pathogenic microorganisms of particular concern to the beef industry by various forms of irradiation has been reported. Regulatory approval of fresh meat irradiation has generated great interest in this cold process technology to inactivate vegetative pathogens in ground beef products and impact positively on their safety. The objective of this study was to determine the inactivation rates of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in frozen ground beef patties as a function of various doses of electron beam irradiation. Fresh ground beef portions were separately inoculated with each pathogen, made into patties and rapidly frozen. The frozen patties were packaged in an irradiation approved barrier film and kept frozen during subsequent irradiation treatments. With the exception of non-irradiated control samples, the frozen patties were irradiated by electron beam at four dose levels (i.e., 1.1, 2.2, 3.3, and 4.4 kGy). Samples were analyzed for the respective pathogens by use of both selective and non-selective plating media. D10 values for *E. coli* O157:H7 in frozen ground beef patties ranged from 0.38 to 0.60 kGy. *Salmonella* D10 values ranged from 0.69 to 1.18 kGy whereas D10 values for *L. monocytogenes* ranged from 0.72 to 1.25 kGy. Low doses (1.1 to 2.2 kGy) of e-beam irradiation were very effective in reducing *E. coli* O157:H7 in frozen ground beef patties and were effective at inactivating *L. monocytogenes* and *Salmonella* but to a lesser extent.

P191 DSC Fate of *Listeria monocytogenes* following Electron-beam Irradiation in Ready-to-Eat Turkey Roll Formulated with Pediocin Alone or Combined with Sodium Lactate and Sodium Diacetate

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The effectiveness of electron beam irradiation for controlling *Listeria monocytogenes* in ready-to-eat (RTE) turkey roll, formulated with pediocin (6,000 or 12,000 AU) alone or combined with sodium lactate (SL, 2.0%) + sodium diacetate (SDA, 0.25%), was investigated. Slices of turkey roll were inoculated with a five-strain mixture of *L. monocytogenes* to give ~ 10^7 CFU/cm^2. Inoculated product without Pediocin or SL + SDA served as controls.

Meat samples were vacuum-packaged then irradiated at 0, 1.0, 1.5, 2.0, and 2.5 kGy. Samples irradiated at 0, 1.5, or 2.5 kGy were stored at 4°C (42 days) or 10°C (30 days). *L. monocytogenes* were enumerated by plating serial dilutions of meat homogenate on Modified Oxford (MOX) agar and counting bacterial colonies on agar plates after incubation (35°C, 48 h). Irradiation at 1.5 and 2.5 kGy reduced initial populations of *L. moncytogenes* by ~ 3.1 and 5.0 log, respectively, irrespective of product formulation. Growth of survivors was completely inhibited in samples with SL + SDA ($P < 0.05$). For example, *L. monocytogenes* in irradiated (2.5 kGy) turkey roll with SL + SDA + pediocin were less than 10^2 CFU/cm^2 throughout storage at 4°C or 10°C. In contrast, survivors in 2.5 kGy-treated samples without SL + SDA reached ~ 10^8 CFU/cm^2 at 20 days (10°C) and 35 days (4°C). Irradiation (2.5 kGy) combined with SL(2.0%) + SDA (0.25%) is effective in reducing *L. monocytogenes* in RTE turkey roll and preventing growth of survivors during refrigeration (4°C) and temperature abuse (10°C).

P192 Withdrawn

P193 Effects of Drying Methods, Gamma Irradiation and Storage on the Carotenoids of Paprika

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The effects of drying methods, gamma irradiation and storage on the carotenoids (capsanthin, capsorubin, zeaxhanthin, capsolutein, violaxanthin, ß-carotene and ß-cryptoxanthin) of paprika were investigated. Sun-dried and dehydrated paprika samples were irradiated in Co 60 gamma irradiator at five doses (0, 2.5, 5.0, 7.5, 10 kGy) in polyethylene bags and stored for ten months at ambient temperature. The individual carotenoid analyses were carried out every two months on the samples and red pepper by reverse phase HPLC. The concentrations of capsanthin and capsorubin, responsible for the atractive red color of the paprika, varied between 524.1 to 1019 mg/kg and 45.38–81.32 mg/kg in sun-dried paprika and 383.5 to 710 mg/kg and 40.72 to 87.00 mg/kg, respectively. Higher irradiation doses and a longer period produced a significant ($P < 0.01$) reduction of all the carotenoids except capsorubin. There was no significant ($P < 0.05$) effect of irradiation doses on capsorubin destruction. The decrease of red carotenoids for all irradiation treatments was less than that related to the storage period. Even the highest irradiation dose, 10 kGy, caused a 10.7% capsanthin reduction, whereas ten months storage at the ambient temperature caused a 49.9% reduction of capsanthin. Yellow pigments of paprika (zeaxhanthin, capsolutein, violaxanthin, ß-carotene, ß-cryptoxanthin) were significantly ($P < 0.01$) decreased by all treatments. The pigments were also found at high levels in sun-dried samples, similar to the finding regarding red pigments. This difference can be explained on the basis of pigment biosynthesis during the sun drying period. The most extensive pigment reduction occurred in processing and storage conditions of paprika rather than in the irradiation process.

P194 Effect of Drying Methods, Gamma Irradiation and Storage on the Capsaicinoids of Paprika

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A study was conducted to investigate the changes in pungent components (capsaicin, dihydrocapsaicin, homodihydrocapsaicin, isodihydrocapsaicin and nordihydrocapsaicin) of paprika as a function of drying method, gamma irradiation and storage period. Sun-dried and dehydrated paprika samples were irradiated by using Co 60 gamma irradiator at five doses (0, 2.5, 5.0, 7.5 and 10 kGy) in polyethylene bags and stored at ambient temperature for ten months. The capsaicinoid contents of the samples were periodically analysed by HPLC every two months during the storage period. The major pungent components were capsaicin and dihydrocapsaicin, at concentrations of 137.7 to 227.6 mg/kg and 79.9 to 137.1 mg/kg, respectively, which increased significantly ($P < 0.01$) with increasing irradiation doses. In contrast, a significant ($P < 0.01$) decrease was observed in these components as the storage period increased. The increases of capsaicin, dihydrocapsaicin and homodihydrocapsaicin content were about 10% with the dose of 10 kGy. In contrast, all three compounds decreased more than 25% during the ten months storage period. The level of all capsaicinoids were significantly ($P < 0.01$) higher in dehydrated paprika than in sun-dried paprika. A small but significant ($P < 0.05$) difference occurred in the amount of homodihydrocapsaicin, one of the minor capsaicinoids in paprika, as the result of irradiation. Nordihydrocapsaicin was solely determined in fresh red pepper, but it was not found in the paprika. Although isodihydrocapsaicin was not detected in paprika during the first five months of the storage period, it appeared after six months. Isodihydrocapsaicin content can be useful for determination of storage time of paprika stored longer than six months.

P195 Evaluation of the VERIcleen™ Food Residue Surface Test as a Means to Monitor Surface Hygiene

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The development of rapid hygiene monitoring methods is a particularly dynamic area of applied food safety research. Over the past decade, time consuming microbiological techniques have, for the routine monitoring of cleaning, been superseded by real-time chemical detection methods. In turn, expensive, instrument-based systems are evolving into a variety of inexpensive, instrument-free tests, each capable of detecting a specific food component. The next stage of this evolutionary process is likely to involve the design and development of single test protocols capable of detecting a combination of component residues. This study evaluates the performance of one such test method—the recently developed VERIcleen™ Food Residue Surface Test (Charm Sciences Inc). To simulate varying degrees of poor cleaning, stainless steel squares (100 cm^2) were inoculated with serial dilutions of different extracts, of foods that are commonly involved in food poisoning outbreaks. The surfaces were sampled using VERIcleen™, immediately after inoculation, while still wet or after they had been allowed to air-dry. VERIcleen™, which primarily detects carbohydrate and phosphate residues, was capable of detecting the presence of raw vegetable homogenates that had been diluted 1000-fold. It was even more effective when used to sample surfaces contaminated with ice-cream and composite manufactured product residues, including cola, orange juice and marmalade. The results also suggested that VERIcleen™ could be used to assess the cleanliness of surfaces that had come into contact with dairy or meat-based products, such as eggs or salami, although with slightly less sensitivity. Neither surface dryness nor the presence of residual sanitizer affected the sensitivity of VERIcleen™ and in all cases, the results obtained were consistent, easy to interpret and attainable within 1 min of sampling the surface. These results demonstrate that VERIcleen™ could be used to assess the cleanliness of food production areas within which a wide range of food types are prepared, for example food service establishments.

P196 DSC Evaluation of Hygiene Training within the Vending Industry

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The quality of vended drinks from vending machines and post-mix dispensers is paramount to consumers and to suppliers of both the equipment and food ingredients. Previous research has shown that effective cleaning of vending machines can be difficult and is militated by time constraints. Proper cleaning has been shown to increase the quality of vended drinks but is dependent on appropriately trained staff. To improve its good hygiene practices and maintain consumer confidence in the products, the vending industry needs specific training programmes. This paper reports the findings of a study examining hygiene training within the vending industry. A training needs analysis (TNA) questionnaire was sent to all vending machine operator companies in the United Kingdom and Northern Ireland. The questionnaire focused on the perceived needs and attitudes towards training within the industry. Using the data generated from the TNA questionnaire, in-depth interviews were conducted in which managers were asked to discuss further detailed aspects of training. In conjunction with the interviews, a questionnaire was given to personnel who were responsible for the cleaning of the machines. The questionnaire explored the operators' training history and their perceptions and attitudes to training and how they felt their employers perceived training. The TNA, interviews and questionnaire data were used to assist in the development of a one-day training workshop for managers. The aim of the workshop was to enable managers to disseminate best practice on cleaning to their personnel responsible for cleaning vending machines. A second training history questionnaire was used to evaluate the success of this process.

P197 From Reactive to Proactive – The Prevention of HACCP Parameters and Related Equipment Failure

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The current HACCP Program is responding to historical failures and makes the necessary corrections to prevent repeated occurrences. A new wireless remote monitoring and control system can prevent failure of HACCP parameters as well as related equipment associated with the process. Moreover, the system constantly records all the data produced and can issue HACCP, quality assurance and production reports, saving substantial labor. The wireless system drastically reduces the cost of plant installation compared to a wired system; furthermore, it can connect to most existing monitoring systems so that no equipment or instrument need to be replaced. The new wireless system can transmit data from numerous plants into headquar-

ters in real time, enabling faster and cheaper central quality assurance supervision of multi plant locations, transport and storage. The headquarter team could also access any plant, transport or storage system and check stored data and the operation of every sensor. The system was designed and tested to monitor various HACCP parameters in shell egg processing plant and its cold storage, which includes storage and processing, temperature, humidity, pH and chlorine level. However, the system can be adapted to monitor HACCP points in various food harvesting and processing facilities.

P198 The Co-relationship between High Technical Food Safety Standards and Operating Cost Effectiveness

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Previous research has indicated a link between efficient process control and hygiene standards and the level of operator hygiene training. This research aimed to determine whether higher technical standards and more highly qualified staff contributed to better processing efficiency and cost effectiveness. A survey of small/medium sized manufacturers was undertaken, using both questionnaires and factory audits, examining labor costs, process line utilization and quality failures/waste control. The results indicated that although there was some correlation with regard to labor costs and line utilization, this linked mainly to the notable improvments in quality, resulting in reduced rework, waste and consumer complaints. The poster presents the findings of this research as a case study outlining the key developments and findings, where customer complaints were reduced by 50% and operational savings of $50,000 per annum.

P199 DSC The Comparison of HACCP Application and Non-application at Food Service Establishments in Korea by Microbiological Hazard Analysis

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The importance of HACCP (Hazard Analysis Critical Control Point) systems has increased at food service establishments in Korea during the last 5 years. Furthermore, the development of commission food service company and performance of P.L. (product liability) are amplified to these concerns. Consumers' demand for food safety has also increased, varying with the improvement in the quality of dietary life. In this paper, we compared HACCP application and non-application at food service establishments in Korea by microbiological hazard analysis. Samples from waterworks, food contact surfaces, surroundings and environment, employees, food items and air at ten food service establishments in Seoul, its vicinities, and Kyeungnam areas, Korea, were evaluated by microbiological analyses such as aerobic plate count (APC), and counts for Coliform group, *E. coli*, *Yersinia enterocolitica*, *Salmonella*, *Staphylococcus aureus*, and *Vibrio parahaemoliticus*. The mean level of APC and Coliform group at the HACCP-instituted foodservices was lower than that of the counterpart group. *E. coli* from sixteen samples and *Staphylococcus aureus* from fifteen samples were isolated, but *Yersinia enterocolitica*, *Salmonella* and *V. parahaemoliticus* were not isolated.

P200 Microbiological Quality Evaluation to the HACCP System of the Bakery Products at Bakeries

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Recently, the HACCP system has been emphasized in processed food industries throughout the world. However, the system has not been applied widely to domestic food industries yet. More studies of HACCP application are needed for settling the system in small-scale bakeries, along with increased consumption of bakery products. This study was performed to determine hazard analysis critical control points (HACCP) at establishments that produce bakery products such as cream, soy paste bread and cakes by Korean food safety regulation article 32. Therefore, the result of this study would make it easy to apply the proper HACCP system and to manage the CCP at bakery product. Two bakery shops located in the Seoul area were selected. Microbial hazard factors in the raw material, baking procedure and working environment of the bakery products were examined. Testing samples were collected under the same conditions. Samples were placed in the sterilizing bottle (125°C, 15 min) and transported in an ice container. Coliforms were found in fresh cream of cake and in soybean paste of soybean paste bread, as much as 10^5 CFU/g in the hazard analysis of raw materials. Furthermore, large numbers of coliforms were detected on the hands of salesmen and bakers. This suggests that CCPs such as fresh cream, soybean paste, and the phase of manufacturing should be strictly managed. In addition, personal hygiene practices should be taught continuously for hygienic bakery products and public health.

P201 Verification of a Food Safety Auditing Tool for Foodservice Establishments Based on Microbiological Analysis

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Foodservice establishments have been identified as the major place for foodborne illness outbreaks in Korea, mainly due to the lack of good sanitary management practices. Verification of a food safety auditing tool developed in this study was done by calculating correlation coefficient between field sanitation auditing scores and microbiological assessment scores in each establishment. The food safety auditing tool developed consisted of nine dimensions and sixty-five items, and its perfect score was 105. Microbiological samples were collected from waterworks, food contact surfaces, non food contact surfaces, hands, food items and air for microbiological analyses such as APC, and enumeration of coliforms, fungi, and *Staphylococcus aureus*. Microbiological data were converted to microbiological assessment scores based on the microbiological guidelines: 2 as acceptable, 1 as marginally acceptable, and 0 as unacceptable. Eight foodservice establishments, including middle/high schools, universities, and employee feedings in Korea were selected for the field auditing and microbiological analysis from June to August in 2002. Among the selected, 5 were appointed as HACCP implemented foodservice establishments by KFDA. Statistical data analysis was completed by use of the SPSS Package, including Spearman's correlation coefficient of nonparametric analysis. Spearman's correlation coefficient value (0.719) between field sanitation auditing scores and microbiological assessment scores showed a significant ($P < 0.05$) correlation, which verifies the fitness of the food safety auditing tool. Continuous self-diagnoses and supervision using a verified food safety auditing tool such as suggested in this study are needed for foodborne illness prevention, especially in foodservice establishments.

P202 DSC Analysis of Critical Control Points through Field Assessment of Sanitation Management Practices in Foodservice Establishments

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Effective sanitation management practices in foodservice establishments are required mostly to prevent foodborne-disease outbreaks. The purpose of this study was to identify critical control points requiring strict control for good sanitary management. Sanitary management practices were assessed using a food-safety auditing tool developed in this study. It consisted of nine dimensions and sixty-five items. The dimensions are personal hygiene, supply of raw food, food storage, separate handling of raw and ready-to-eat foods, serving, cleaning and sanitizing, waste control, pest control, and sanitation control of facilities and equipment. Field assessment was conducted at twenty foodservice establishments in Korea from June to August in 2002. Statistical data analysis was completed using the SPSS Package. The average auditing score was 74.3 out of 105. The average number of in compliance items was 43.9 out of 65 and out of compliance was 18.9. The three highest percentages of out of compliance were for the dimensions of waste control, sanitary control of facilities and equipment, and supply of raw food materials. The highest percentage of out of compliance in the sixty-five items revealed as prohibition of placing raw food materials on the floor in receiving (75%), followed by clean condition of refrigerators (65%), installation of hot/cold holding equipment at service (55%), and monitoring of heating/reheating temperature (45%). In order to enhance the managerial control of food sanitation, foodservice managers should pay more attention to education and trainings for reduction of out of compliance observations.

P203 Usage Status Survey on Some Essential Facility, Equipment and Documentary Records for HACCP Implementation in Contracted Foodservices

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With the increasing recognition of the importance of sanitary management, many Korean foodservices are voluntarily implementing HACCP systems. Moreover, along with amendment of the Food Sanitation Act in December 2000, KFDA (Korean Food and Drug Administration) started to appoint foodservices with HACCP application. In preceding studies, the most troublesome factors in HACCP application have been the lack of proper facility and equipment. Therefore, in this study, based on HACCP prerequisite program in Food Sanitation Act, essential facility, equipment and documentary records will be identified. Usage and adoption of these devices at KFDA appointed HACCP foodservices (Appointed), voluntary HACCP applying foodservices (Voluntary) and HACCP non-applying foodservices (Non-applying) have been compared. A total of 46 contracted foodservices were surveyed: 13 Appointed (65% of nation-wide all appointed), 17 Voluntary and 16 Non-applying. For usage and adoption of facility and equipment,

11 out of total 20 surveying items showed significant differences at the three foodservice groups ($P < 0.01$). Specifically, the following items showed lower usage: partition off in the kitchen, draining related items, thermometer installation in refrigerator and freezer. Regarding CCP monitoring equipment installation, 7 out of 8 items showed significant differences among the groups ($P < 0.01$ or $P < 0.05$). For the usage of 10 documentary recording items for HACCP application log, 8 items showed significant differences among the groups ($P < 0.01$ or $P < 0.05$). Especially, the air-borne bacteria count log was the lowest used item except the Appointed. Resultantly, those essential facility, equipment and documentary records were used to a small extent only in the Appointed. This limited usage was also true for the Voluntary where the dietitian answered they applied HACCP voluntarily. This demands further improvement.

P204 Efficacy Quenching of Chlorine Dioxide and Quaternary Ammonium-containing Sanitizers by Organic Matter

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The effect of organic matter on the efficacy of two sanitizers containing chlorine dioxide (CD) or a twin-chain quaternary ammonium compound (QAC) blend was evaluated in vitro against a dairy-associated *Bacillus* (B.) *cereus* strain. Planktonic *B. cereus* DL5 cells were grown in Tryptone Soya Broth (TSB) for 24 h and harvested at 5000 rpm. Cells were then resuspended in 20 ml of either TSB or sterile distilled water (SDW) to simulate presence or absence of organic matter, respectively. Cell suspensions were then treated with CD or QAC, at concentrations recommended by the manufacturer, for 1 min. Cells treated with SDW instead of sanitizers served as controls. After neutralization, *B. cereus* DL5 cells were enumerated on Tryptone Soya Agar by the droplet plate technique. Counts of *B. cereus* DL5 cells in untreated cell suspensions were ca. 8 log CFU/ml. For *B. cereus*, DL5 cells resuspended in SDW, log reductions were significantly ($P < 0.05$) higher than for *B. cereus* DL5 cells resuspended in TSB when treated with CD. However, log reductions of *B. cereus* DL5 cells resuspended in either SDW or TSB and treated with QAC were not significantly different from each other. Results suggested that organic matter quenched the efficacy of the CD-containing sanitizer used in this study against *B. cereus* DL5 cells, while the efficacy of the QAC remained largely unaffected.

P205 DSC Sandia National Laboratories Decon Foam-100 as a Sanitizer against *Listeria monocytogenes* Mixed Culture Biofilms

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Food processors of ready-to-eat (RTE) foods are facing issues with contamination from *Listeria monocytogenes* that may occur due to biofilms present on various food contact surfaces found throughout the processing environment. These microorganisms are able to survive the cleaning and sanitizing regimen due to increased resistance mechanisms. The Decon Foam-100 (DF-100) was developed for use against resistant *Bacillus anthracis* spores. The DF-100 is a two-part formulation containing quaternary ammonium compounds and 1% hydrogen peroxide. The DF-100 was used in this study as a highly effective sanitizer against *Listeria monocytogenes* and *Pseudomonas putida* containing biofilms. The biofilms were developed on five various food contact surfaces for 14 days and were treated after 24 h, 72 h, 7 days, and 14 days. The treatment included exposure to the DF-100 for 1 min, 5 min, and 10 min. All samples were neutralized to ensure control of exposure time. Samples were enumerated using modified Oxford medium (Oxoid, UK) and Tryptic Soy Agar (TSA, Difco). A GLM Procedure in SAS was used for statistical analysis comparing differences in the Least Squares of Means for biofilm age, surface, and treatment. Enumeration resulted in 5.52 to 7.87 log CFU/cm^2 *L. monocytogenes* and 4.34 to 6.55 log CFU/cm^2 *P. putida* from 24 h to 14 days over all test surfaces, respectively. The DF-100 resulted in 4 to 6 log reductions for all treatment times. Overall resistance to the sanitizer increased with biofilm age for both *L. monocytogenes* and *P. putida* on all five test surfaces, $P \leq 0.05$.

P206 Recovery of *Listeria monocytogenes* and *Pseudomonas putida* from Food Contact Surfaces after Ozone Exposure

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The effect of 1 and 5 min exposure to ozone was analyzed on a 24 h biofilm of *Listeria monocytogenes* and *Pseudomonas putida* grown on 10 cm^2 test coupons of Nylon (NYL), Buna-n Nitrile Rubber (BUN), Stainless Steel type 304 #2b finish (STS), and Ultra High Molecular Weight polyethylene (POL). Ozone was applied inside an experimental ozone system engineered by Wenger Manufacturing and BOC gases, using a PCI-WEDCO model HC-500 (Environmental Technologies, W. Cadwell, NJ). After ozone exposure, *L. mono-*

cytogenes counts were recovered using Modified Oxford Media (MOX) and Tryptose Soy Agar (TSA). *P. putida* counts were recovered using TSA. Reduction differences among surfaces were compared to population counts on non-treated inoculated coupons. For *L. monocytogenes*, the initial counts ranged from 5.8 to 6.8 log CFU/cm^2. Recoveries were not different in TSA when compared to MOX in all food contact surfaces ($P > 0.05$). After 1 min exposure, ozone reduced counts by 0.6, 0.7, 1.0 and 1.3 log CFU/cm^2 for STS, POL, NYL and BUN, respectively. After 5 min exposure, reduction levels were not significantly different ($P > 0.05$) over those achieved in the 1 min exposure treatment. For *P. putida*, initial counts ranged from 2.4 to 4.4 log CFU/cm^2. After 1 min exposure, ozone reduced counts by 0.3, 2.1, 2.1 and 2.5 log CFU/cm^2 for STS, POL, NYL and BUN, respectively. After 5 min exposure, reduction levels were not significantly different ($P > 0.05$) from those achieved in the 1 min exposure treatment.

P207 A Comparison of Attachment and Recovery Methods for Microorganisms Attached on Various Food Contact Surfaces

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Biofilm formation using a rotating disk reactor (turbulent conditions) was examined using *Pseudomonas aeruginosa, Pseudomonas fluorescens, Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* Typhimurium on various coupon surfaces. Each organism was grown on stainless steel, glass, polycarbonate and polyvinyl chloride (PVC) coupons for 48 h in a reactor and sampled with calcium alginate swabs. Biofilm formation (static conditions) and recovery were also examined using coupons of stainless steel, high-density polyethylene (HDPE), polycarbonate, and PVC submerged in tryptic soy broth with yeast extract inoculated with one of the above bacteria, and compared with that of the rotating disk reactor. After 48 h, the coupons were sampled with cotton and calcium alginate swabs. The method of recovery was also studied by inoculating coupons of stainless steel, HDPE, polycarbonate and PVC, drying them in a laminar hood, and sampling after 48 h with cotton and calcium alginate swabs. Using the reactor method, 10^8 CFU/coupon surface was recovered for *P. aeruginosa* while 10^5–10^6 CFU/coupon surface were recovered for the other organisms. Recovery with the immersion method was 10^8–10^9 CFU/coupon surface with cotton swabs and 10^7–10^9 CFU/coupon surface with calcium alginate. With the non-immersion method, the cotton swab recovered 10^4–10^6 CFU/coupon surface, and the calcium alginate recovered 10^3–10^5 CFU/coupon surface. *P. aeruginosa* formed equally good biofilms under static and turbulent conditions while the other bacteria formed better biofilms under static conditions. Recovery was better with cotton swabs than with calcium alginate swabs, and this may be due to the calcium alginate fibers disintegrating and sticking to the coupon surface.

P208 Comparison of Cell Attachment and Spore Formation by *Bacillus cereus* DL5 in Minimal Nutrient Growth Medium

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It has recently been suggested that spore formation plays an integral role in biofilm formation by *Bacillus* (B.) *subtilis*. However, no corresponding data exist for spore formation and cell attachment by *B. cereus*. This study evaluated spore formation in minimal nutrient medium by vegetative cultures of *B. cereus* DL5, either attached to glass wool, or in planktonic form harvested from flasks containing glass wool (PGW), or in planktonic form harvested from flasks without added glass wool, at 9 time intervals over 48 h. Total vegetative cell plus spore counts were determined by plate counting using the droplet plate technique on Standard One Nutrient Agar (SONA) and incubation at 37°C. Spore counts only were determined after heating sample aliquots at 80°C in a waterbath for 10 min and pour plating in SONA re-inforced with 1.5% Commercial Agar. Results indicated that the highest spore counts were obtained in the attached cultures (5.26 log CFU/ml), followed by PGW (4.15 log CFU/ml) and planktonic (3.70 log CFU/ml) cultures. The presence of attachment surfaces thus apparently stimulated spore formation in vegetative cultures of *B. cereus* DL5 growing in minimal medium. These results may have important implications for food processors as *B. cereus* DL5 cells appear not only to attach to food processing equipment surfaces, but also perhaps to be stimulated to form spores in parallel with cell attachment. Since spores of *Bacillus* spp. are more tolerant to cleaning and sanitation regimes than are corresponding vegetative forms, they are likely to pose added risks of product contamination.

P209 Bacterial Contamination of Commercial Yeast

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This study evaluated a commercial yeast manufacturing process for contamination with *Enterococcus*, coliforms and *E. coli*. Five replicate surveys of a commercial yeast manufacturing process and 2 replicate surveys of a seed yeast

manufacturing process were carried out. Samples were taken upstream from final compressed and dry yeast products at 7 points along the production lines. Yeast samples were analyzed before and after preliminary incubation (PI), at 37°C for 24 h by standard methods on selective media using the pour-plate technique. The PI procedure was incorporated to allow for detection of bacterial counts which were below the lower detection limit (0.7 log CFU/ml) in corresponding non-PI samples, in particular *E. coli.* Presence at all stages and progressive increases in *Enterococcus*, coliforms and *E. coli* counts during processing in the commercial manufacturing operation suggested that the primary source of contamination of both compressed and dry yeast with *Enterococcus*, coliforms and *E. coli* occurred during seed yeast manufacturing but was amplified throughout the commercial yeast manufacturing process to the finished product. This was confirmed by surveys of the seed yeast manufacturing process, which indicated that contamination of seed yeast with *Enterococcus*, coliforms and *E. coli* occurred during scale up of seed yeast biomass to serve as inoculum for commercial fermentation. In the commercial yeast manufacturing process, the commercial fermentation stage, storage of liquid yeast and packaging clearly contributed to increased counts of *Enterococcus*, coliforms and *E. coli.*

P210 Assessment of Bacterial Populations on Equipment Surfaces in a Processed Meat Slicing Operation by Different Techniques

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Microbial populations associated with surfaces on processed meat slicing equipment were quantified and characterized from 3 sites along each of the modified atmosphere (MAP) and vacuum packaging lines (VACPAC) of a small scale processed meat factory. Mock stainless steel surfaces were attached to each sampling site and detached after 7, 14, 21 or 28 days. Attached cells were dislodged and enumerated. At the same time intervals, equipment surfaces were also swabbed. Aerobic plate counts (APC) and Gram-negative counts (GNC) were determined and the predominant bacterial populations isolated and characterized. Cell attachment to mock surfaces was also assessed by use of scanning electron microscopy (SEM). While variable results were obtained by the swab technique, the mock surface technique and SEM indicated increased bacterial accumulation and attachment, respectively, over time. Overall, the highest APC were obtained from the VACPAC line using both the swab (4.26 log CFU/ml) and mock surface (4.74 log CFU/ml) technique. The GNC remained below 2 log CFU/ml throughout the study. Characterization of predominant isolates obtained by both the swab and mock surface technique indicated that the populations associated with equipment surfaces were predominantly Gram-positive (85%) and consisted of *Staphylococcus, Micrococcus, Bacillus*, and lactic acid bacteria (in decreasing order).

P211 Inactivation of GFP-transformed *Escherichia coli* O157:H7 on Whole Apples following Immersion in Selected Chemical Sanitizers at 25°C and 55°C

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The viability of GFP-transformed *E. coli* O157:H7 (strain B6-914) on whole apples was evaluated in response to a two-min exposure to distilled water (control) and five different sanitizers – sodium hypochlorite (200 ppm chlorine), 5% hydrogen peroxide (H_2O_2), sodium bicarbonate buffer ($NaHCO_3$, pH 11.5), 80 ppm Tsunami100®, and 1.5% H_2O_2 +1.5% lactic acid. The stem area of unwaxed Red Delicious apples, spot-inoculated with *E. coli* O157:H7, was immersed in distilled water or sanitizers at 25°C or 55°C for 2 min, and rinsed for 5 s in fresh distilled water. Survivors were enumerated by vigorously washing apples in Buffered Peptone Water (BPW), surface plating samples of BPW onto Sorbitol MacConkey Agar (SMA) and counting bacterial colonies on SMA plates after incubation (37°C, 24 h). Numbers of *E. coli* on apples were ~ 5.26 $\log_{10}$ CFU/apple before immersing in water or sanitizers. $\log_{10}$ reduction in numbers of *E. coli* on apples following immersion in water, chlorine, H_2O_2, $NaHCO_3$, Tsunami100®, and H_2O_2 + lactic acid at 25°C were 1.71, 1.87, 2.55, 2.47, 1.99, and 3.35, respectively. Increasing the temperature to 55°C enhanced the antimicrobial effectiveness of the sanitizers. $\log_{10}$ reductions at 55°C increased to 1.73, 1.91, 2.66, 2.56, 2.44, and 3.51 after treatment with water, chlorine, H_2O_2, $NaHCO_3$, Tsunami100®, and H_2O_2 + lactic acid, respectively. The use of 1.5% H_2O_2 + 1.5% lactic acid at 25°C or 55°C seems to have good potential as an effective treatment for destroying *E. coli* O157:H7 on whole apples.

P212 DSC Optimization of Chlorine Treatments and the Effects on Survival of *Salmonella* spp. on Tomato Surfaces

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In the past 10 years, foodborne disease outbreaks due to bacterial pathogens on fresh produce have risen dramatically. Salmonellosis is the most

commonly reported foodborne bacterial infection in the United States, whereas gasteroenteritis caused by *Campylobacter* likely causes the highest estimated number of cases. *Salmonella* has been implicated in several outbreaks involving tomatoes. The most critical point in proper sanitation of tomatoes is water quality and the maintenance of chlorine, pH and temperature of water. Tomatoes are currently rinsed in chlorine at 150 to 200 mg/L and pH 6.5 to 7.5 at 10°C above pulp temperature. Various other approved sanitizers are used and studied on fresh produce commodities, including hydrogen peroxide, acidified sodium chlorite, ozone, chlorine dioxide and peroxyacetic acid. This study was intended to determine the optimum sanitizer for removal of *Salmonella* from tomato surfaces. Inoculated *Salmonella* was reduced by more than 5.0 $\log_{10}$ units by 150 mg/L of chlorine at pH 6.5 after 1 min. *Salmonella* was not eliminated from the tomato surfaces. In a wound study at 25°C and 35°C with the same parameters, there was only a 1.0 $\log_{10}$ reduction in *Salmonella* populations. Acidified sodium chlorite, peroxyacetic acid and hydrogen peroxide demonstrated a reduction of 5.0 $\log_{10}$ units after 30 s. Ozone, peroxyacetic acid and chlorine dioxide had similar effects to those of the aforementioned chlorine treatment. Sanitizers proven effective and economical in the removal of *Salmonella* will assist in establishing definitive guidelines in pathogen reduction on tomatoes for regulators, processors, and packinghouses in the US.

P213 Meta-analysis of the Microbiological Quality of Food in Relation to HACCP and Food Hygiene Training in Food Premises in the United Kingdom, 1997–2002

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Meta-analysis of eight UK food studies was performed to determine the microbiological quality of food and the extent to which food premises comply with legal requirements (presence of a hazard analysis system, food hygiene training). Of the 19,022 premises in these studies, two-thirds (66%) were catering premises and 34% were retail premises. Significantly more ready-to-eat food samples from catering premises (20%; 2,511/12,703) were of unsatisfactory or unacceptable microbiological quality compared to those from retail premises (12%; 1,039/8,462) ($P < 0.00001$). Food hygiene inspections (Local Authority Inspectors' confidence in management; consumer at risk scores) indicated that poor microbiological quality was associated with little or no confidence in the food business management of food hygiene, and small premises. Three-quarters (76%) of retail premises had hazard analysis in place compared with 59% of catering premises ($P < 0.00001$). In most (87%) retail premises the manager had received some form of food hygiene training, compared with 80% of catering premises ($P < 0.00001$). Retail premises where the managers had received some form of food hygiene training were more likely to have a documented hazard analysis system in place (77%) compared to managers of catering premises that had received food hygiene training (63%) ($P < 0.00001$). The meta-analysis suggests that the lower microbiological quality of ready-to-eat foods from catering premises, compared with those from retail premises, may reflect differences in management food hygiene training and the presence of a hazard analysis system. The importance of adequate training for food handlers and their managers as a pre-requisite for effective HACCP based controls is recognized.

P214 The Microbial Ecology of High Risk, Chilled Food Factories; Evidence for Persistent *Listeria* spp. and *Escherichia coli* Strains

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A three year assessment of the microbial ecology of the high risk processing areas in five UK chilled, ready-to-eat food factories was undertaken. The project's objectives were to investigate if there was any evidence of microbial strain persistence and to identify whether strains of selected species adapt to specific environmental or product niches. Approximately 196,000 and 75,000 product and environmental samples were examined for *Escherichia coli* and *Listeria*, respectively. Isolation was by traditional methods and strain identification was by ribotyping. Of 154 *E. coli* isolates ribotyped, 44 from the environment and 110 from food products, 121 isolates, in 63 Ribogroups, were positively identified as *E. coli*. Of 279 *Listeria* spp. isolates identified and ribotyped, 269 isolates, 183 from the environment and 86 from food products, in 31 Ribogroups, were positively identified as *Listeria* spp. The overall incidence of *E. coli* and *L. monocytogenes*, at 0.08% and 0.24% respectively, was very low and illustrated the good hygiene controls practised by the five factory sites. Some 10 *E. coli* Ribogroups showed evidence for persistence, being recovered for between 1 and 15 months with a mean of 5.6 months. One Ribogroup dominated: Ribogroup 102-248-S-4 was found on 54 occasions in three sites, and was the most persistent (15 months). Some 14 *Listeria* spp. Ribogroups showed evidence for persistence, being recovered for between 2 and 25 months with a mean of 13.8 months. Again, one Ribogroup dominated: Ribogroup 102-195-S-1 was found on 82 occasions, was present in all five sites and was very persistent (21 months). The majority

of *E. coli* strains were product niche oriented while the majority of *Listeria* spp. strains were environmental niche oriented. The two dominant *E. coli* and *L. monocytogenes* strains, however, were present in both niches. The majority of the *Listeria* spp. isolates (64.7%) were identified as *L. monocytogenes*, followed by *L. innocua* (23.8%), *L. welshimeri* (6.3%) and *L. seelgeri* (5.2%). *Listeria* spp. strains differed primarily in their isolation rate, isolate to Ribogroup ratio and environmental or environmental/product colonization niche basis. This is the largest study known of its type and clearly indicates that *E. coli* and *L. monocytogenes* can become persistent for extended time periods, even in the best designed and operated food factories. The implications of these findings in terms of sampling and factory controls will be discussed.

P215 Sanitary Standard Operation Procedures in a Tortilleria at Xalapa, Veracruz, México

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In Mexico, the consumption of tortilla is about 300 g daily per person, amounting to a total of 19.7 millions of tons per year. For most of the population, it represents about 70% of their diet. In 2001 and 2002, many tortilla companies were closed because the Secretaría de Salubridad y Asistencia (SSA) laboratory detected high levels of coliforms and non hygienic practices. The main purpose of this study consisted in assessing one of the biggest companies of tortillas in Xalapa, Veracruz to diminish the problems associated with critical control points (CCP) identified by SSA, to improve hygienic practices according to the established regulations, and to implement the Sanitary Standard Operation Procedures (SSOP). From the 90 points evaluated at the first visit by SSA, 61% were detected as CCP violations. Certified personal from LATEX evaluated the tortilleria and proposed some corrective actions that were established in less than five months. For the second visit, there were only 22% CCP violations as detected in document reports. By now, the company is implementing the use of logbooks for each area, good hygienic practices for workers, disease control, some details in the infrastructure and elaborating the SSOP manual. This is the first descriptive study in the implementation of SSOP in tortilla production for Veracruz State that demonstrates that people are becoming aware about the importance of improving quality controls in their establishments.

P216 The Increased Effectiveness of Peracetic Acid with a Foaming Additive on Fungal and Bacterial Spores

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Peracetic acid (PAA) has been widely used in CIP applications in the food industry because of its excellent biocidal properties and user-friendly product attributes. Generally, peracetic acid is used at concentrations exceeding several hundred ppm when treating mold and bacterial spores. Such high levels are often undesirable because of the increased odor, irritation and corrosivity associated with the higher concentrations. Laboratory studies were performed to determine if peracetic acid combined with a foaming additive would be more effective at lower concentrations in treating spores than peracetic acid alone. Initial studies were done using a *Bacillus subtilis* spore suspension and ISO-GRID methodology. Test solutions of 1000, 1800 and 2600 ppm PAA were prepared with and without foaming additive. The solutions with additive were applied as a liquid rather than foam to determine the effects of the combined formulation without the additional variable of foaming. Each solution was tested in triplicate, with a contact time of 60 s. Three untreated positive control filters were also included. The $\log_{10}$ recovery from each test was: Positive control = 4.88; 1000 ppm PAA = 3.84; with foaming additive = 0.00; 1800 ppm PAA = 1.71; with additive = 0.00; 2600 ppm PAA with and without additive = 0.00. It is apparent that there is a significant increase in the efficacy of peracetic acid at lower concentrations when the additive is used. This increase in efficacy can in part be attributed to the increased surfactancy and decreased surface tension, allowing for more efficient penetration of peracetic acid through the spore coat.

P217 Altered Sensitivity of Acid and Cold Adapted *Listeria innocua* to the Quaternary Ammonium Compound Cetrimide

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Microorganisms have genetic and physiological means to ensure survival of sub-lethal levels of the stress. These stress response mechanisms enable the microorganism to persist in environments that would otherwise result in destruction of the organism. To assure destruction of microorganisms, chemical sanitizers are typically applied following cleaning to food manufacturing equipment and the environment. Quaternary ammonium compound sanitizers destroy microorganisms following adherence to the microbial cell, resulting in release of intracellular metabolites. Our hypothesis is that *Listeria innocua*, adapted to acid and cold condi-

tions, has altered sensitivity to the quaternary ammonium compound benzalkonium chloride (cetrimide). To test our hypothesis, *Listeria innocua* was exposed to 10-ppm cetrimide following adaptation to acid and cold conditions. Adaptation to acid was accomplished by incubating *Listeria innocua* in tryptic soy broth containing 1% glucose overnight at 37°C (final pH 4.7). *Listeria innocua* was adapted to cold by incubating washed overnight culture in tryptic soy broth for one week at 10°C. Acid adapted *Listeria innocua* had diminished sensitivity to cetrimide while cold adapted had enhanced sensitivity compared with non-adapted controls. These data indicate microorganisms have altered sensitivity to quaternary ammonium compounds upon adaptation to acid and cold conditions and that adaptation to acidic food manufacturing environments may enable microbial persistence.

P218 Removal of *Pseudomonas putida* Biofilm and Associated Extracellular Polymeric Substances from Stainless Steel by Use of Alkali Cleaning

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Alkali cleaning solutions are commonly used in the food industry to clean food contact surfaces. However, little information is available on the ability of alkali (NaOH) to remove extracellular polymeric substances (EPS) produced by biofilm bacteria. The objective of this study was to determine the NaOH concentration and temperature necessary to remove biofilm EPS from stainless steel under turbulent flow conditions (clean-in-place simulation). Biofilms were produced by growing *Pseudomonas putida* on stainless steel for 72 h at 25°C in a 1:10 dilution of trypticase soy broth. The biofilm-containing coupons were cleaned, using NaOH concentrations from 1.28 to 6.0% and temperatures ranging from 66 to 70°C. Removal of EPS was determined by direct microscopic observation of samples stained with fluorescent-labeled PNA lectin. Treatment with 1.26% alkali at 66°C for 3 min was insufficient to remove biofilm EPS. Treatments with 2.5% NaOH at 66°C and 2.0% NaOH at 68°C for three min were effective at EPS removal. Control of cleaning solution temperature is critical for achieving complete removal of biofilm EPS in CIP systems.

P219 Outbreak Alert!: A Compilation and Analysis of Food-Poisoning Outbreaks

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Contaminated foods are estimated to cause 5,000 deaths and 76 million illnesses in the U.S. per year. The Center for Science in the Public Interest (CSPI) maintains a database of foodborne-illness outbreaks categorized by food vehicle. Linking outbreaks to specific foods serves to alert consumers to food-safety hazards and gives policymakers and public-health officials better information to design risk-based hazard-control plans. CSPI's database was compiled from sources including the Centers for Disease Control and Prevention (CDC), state and local health departments, and medical and scientific journals. The database is updated yearly, and contains only those outbreaks with known or suspected etiology and food vehicle. CSPI found that the top five food categories linked to outbreaks (2,472 outbreaks), not including multi-ingredient foods, were seafood (539 outbreaks), produce (293 outbreaks), eggs (277 outbreaks), beef (251 outbreaks), and poultry (235 outbreaks). Multi-ingredient foods, including salads, pizza, and sandwiches, were linked to 330 outbreaks. Overall, 27% of food-poisoning outbreaks were attributed to meats such as beef, poultry, pork, luncheon meats, and game, while 66% of outbreaks were linked to other foods such as seafood, multi-ingredient foods, eggs, produce, dairy, breads, and beverages, and seven percent of outbreaks were linked to multiple foods. Historically, meats have been thought to pose greater risks than other foods because of possible contamination with pathogenic microorganisms that can live inside animals' intestines. As these data emphasize, all types of foods have the potential to carry hazards and should be treated properly and handled safely to avoid food-poisoning outbreaks.

P220 Factors That Influence the Efficacy of Risk Communication and Consumer Perceptions of Sources of Food Safety Education

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Effective food safety education is required to reduce the incidence and risk of foodborne disease. The development of an effective food safety education strategy is considered to be a complex process and better education depends upon a better understanding of the modes and channels of communication that people actually use. Therefore, factors that influence the efficacy of risk communication and consumer perceptions of food safety education sources have been investigated. A postal, self-complete questionnaire was distributed to a cross-section of the population (response rate 61%). The questionnaire assessed consumer receptivity towards food safety education, perceptions of leaflets and television as sources for food safety information, preferences for all food safety educational sources and an assessment of spokespersons and organizations perceived to provide credible and trustworthy information. Overall, results showed that consumer receptivity to food safety education was positive; however, significant differences

($P < 0.05$) between respondent demographics were identified, thus suggesting the need for different educational strategies for targeted audiences. Organisations associated with the most trust and credibility were determined as Environmental Health Departments and the Food Standards Agency (UK). Similarly, the most believable spokespersons for promotion of food safety information were determined as Environmental Health Officers and the Chief Medical Officer. The most preferable sources of food safety information were determined as food packaging, followed by advice from a medical doctor, leaflets and television. Provision of information from preferable sources, and sources that are perceived to be credible and trustworthy, may increase source effectiveness. Overall, findings from this study provide valuable information for the development of effectual, targeted food safety education initiatives that may increase the potential effectiveness of future food safety education initiatives.

P221 The Cost Effectiveness of a Targeted Disinfection Program in Household Kitchens to Prevent Foodborne Illnesses in the United States, Canada, and the United Kingdom

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Foodborne illnesses impose a substantial economic and quality-of-life (QOL) burden on society due to acute morbidity and chronic sequelae. We developed an economic model to evaluate the potential cost effectiveness of a disinfection program that targets high-risk food preparation activities in household kitchens. For the U.S., Canada, and the U.K., we used published literature and expert opinion to estimate the cost of the program; the number of cases of *Salmonella*, *Campylobacter*, and *Escherichia coli* O157:H7 infections prevented; and the economic and QOL outcomes. In our primary analysis, the model estimated that approximately 80,000 infections could be prevented annually in U.S. households, resulting in $138 million in direct medical cost savings (for example, physician office visits and hospitalizations avoided), 15,845 quality-adjusted life-years (QALYs) gained, $788 million in program costs, and a favorable cost-effectiveness ratio of $41,021/QALY gained. Results were similar for households in Canada and the U.K. (CDN $21,950/QALY gained and £86,341/QALY gained, respectively). When we evaluated implementing the program only in U.S. households with high-risk members (those less than 5 years of age, greater than 65 years of age, or immunocompromised), the cost-effectiveness ratio was more favorable ($10,163/QALY gained). Results were similar for high-risk households in Canada and the U.K. (CDN $1,915/QALY gained and £28,158/QALY gained, respectively). We conclude that implementing a targeted disinfection program in household kitchens in the U.S., Canada, and the U.K. appears to be a cost-effective strategy, falling within the range generally considered to warrant adoption and diffusion (<$100,000/QALY gained).

P222 Consumer Attitudes and Perceptions towards Food Safety in the Domestic Kitchen

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The domestic kitchen is an important point of origin for incidence of foodborne disease and food safety education is required to improve food-handling behaviors in the home. A key to the design of effective educational initiatives is an understanding of factors that influence food safety behaviors. Attitudes are important determinants of food safety behaviors and therefore determination of consumer attitudes towards food safety in the domestic kitchen is important for development of food safety educational initiatives. A self-complete questionnaire was self-administered to consumers representing a cross-section of the population (n=100). Results showed that attitudes towards key food safety behaviours such as hand-washing and drying, cross contamination, cooking and storage were positive; however, attitudes towards cooling principles required improvement. Significant correlations ($P < 0.05$) between attitudinal responses and demographics were identified, suggesting that targeted educational strategies are required for different groups of consumers. Findings denoting perceived personal risk for foodborne illness and personal control and responsibility for food safety demonstrated judgements of optimistic bias and the illusion of control. The majority of consumers (90%) considered themselves to have a low risk of illness after food preparation and 71% consumers perceived the home to be the most unlikely source of foodborne illness. Such perceptions may prevent consumers from taking appropriate steps to reduce exposure to food-related hazards during domestic food preparation. Furthermore, perceived personal invulnerability and failure to associate the home with foodborne disease may hinder educational efforts. Results of this study will be discussed within the context of development of educational initiatives designed to raise awareness of food safety issues and bring about behavioural improvement.

P223 Influence of Fingernail Length and Type on Removing Feline Calicivirus from the Nail Regions Using Different Hand Washing Interventions

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Inadequate hand washing has been a contributing factor for many outbreaks of foodborne illness, and areas underneath fingernails harbor greater microbial populations than other areas of the hand. An evaluation of the efficacy of different hand washing intervention to remove microorganisms from different types (artificial vs. natural) and lengths of nails is needed. Volunteers with artificial or natural fingernails were recruited and their fingernail lengths were measured. Volunteers' nails were contaminated with artificial feces containing feline calicivirus (FCV). They then washed their hands and nail regions with tap water, liquid soap, antibacterial liquid soap, alcohol gel, liquid soap plus alcohol gel, or liquid soap plus a nailbrush. FCV on nails was recovered by scrubbing fingernails with an electronic toothbrush soaked in Eagle essential medium before and after handwashing. FCV populations were determined by tissue culture infective dose (TCID50). The greatest reduction was obtained by washing with liquid soap plus a nailbrush (2.5 $\log_{10}$/volunteer). The least reduction was obtained from rubbing with alcohol gel (less than 1 $\log_{10}$/volunteer). Lower, but not significant ($P > 0.05$), reductions of FCV populations were obtained from artificial than natural fingernails. However, significantly ($P < 0.05$) higher FCV populations were recovered from artificial nails than natural nails before and after handwashing. In addition, FCV populations were correlated with fingernail length, with greater numbers beneath longer fingernails. These results indicate that the best practices for fingernail sanitation for food handlers are to maintain short fingernails and scrub nails with soap and a nailbrush.

P224 Withdrawn

P225 Molecule Cloning, Expressing, and Characterization of a Recombinant Antibody against Sulfamethazine

DSC

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In this study, a recombinant single chain variable fragment (scFv) antibody specific for sulfamethazine (SMZ) was cloned using mRNA from an existing hybridoma cell line 1H11 that produced anti-SMZ monoclonal antibody. The heavy-chain and kappa light-chain variable region genes were isolated by RT-PCR and joined by a DNA linker encoding peptide (Gly_4Ser) as a single-chain scFv DNA. The scFv DNA fragment was cloned into a phagemid (pCANTAB 5E) and expressed as a fusion protein in *E. coli* TG1 or a soluble protein in *E. coli* HB2151. The expression of scFv recombinant antibody was analyzed by SDS-polyacrylamide gel electrophoresis and Western blot. The molecular weight of recombinant antibody is approximately 30-KDa. Nucleotide sequence analyses revealed that the VH gene is about 363 bp long, encoding 121 amino acids, and the VK gene is about 333 bp long, encoding 111 amino acids; they have high sequence similarity with the variable domains of mouse antibody genes that have been published. The deduced amino acid sequences of VH and VK contained four FRs, three CDRs and two cysteine residues necessary for maintenance of the antibody structure, indicating that they were functionally rearranged. The location of the expressed antibody was found to be in the cytoplasm as well as the periplasm. The optimum cell culture conditions for expression recombinant antibody in *E. coli* HB2151 are incubation at 30°C for 6 h after IPTG induced. The affinity of soluble scFv antibody was detected by ELISA. It was proved that the recombinant antibody has specificity as high as that of its parent monoclonal antibody.

P226 Assessment of Mutagenicity and Carcinogenicity Effects of Plastic Bags and Disposable Food Containers in the *Salmonella*/Microsome Test

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Nowadays, plastic bags and disposable food containers made of high and low-density polyethylene have found increasing use in Iran. As there is a direct relation between the safety of such a type of product with the society's health, in this research, the mutagenicity and carcinogenicity of these products have been investigated using Ames test and *Salmonella* Typhimurium (TA100, TA104). As the result of mutation, these bacterial strains have no potential to produce histidine upon being cultured in a minimal glucose agar medium. In general, carcinogenic materials will cause a reverse mutation of these bacteria and results in production of histidine by the bacteria in the MGA medium for the Ames test. To investigate the mutagenicity of the PE bags and containers, we faced the pieces of these products on a test plate *S.* Typhimurium (TA100, TA104) and the results of the experiments were then compared with MGA medium containing

sodium azide as the positive control and distilled water as the negative control. The comparison of the colonies in the negative control, which are produced spontaneously, with those of the investigated PE samples provide a way to prove the mutagenicity of the experimental samples. Results showed that the HDPE grades as the raw material and their products made in the form of such items as disposable glasses and containers, drinking bottles and milk bags don't possess the mutagenicity property. The thin film as plastics bags caused reverse mutation of *S.* Typhimurium of these products. If these products were coated by liquid or solid oils they were found to have much greater effects on mutagenicity of the films. Following the above tests, rat liver tissue microsomes were later obtained under sterile conditions and added to a MGA medium containing the suspected PE samples. The results obtained from the latter experiments confirmed that the PE thin bags used for packaging of the food products in Iran are carcinogenic.

P227 Using a Viral Symbiont to Evaluate Water Samples for the Presence of Viable *Cryptosporidium parvum* Oocysts

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Waterborne transmission of *Cryptosporidium parvum* oocysts has been implicated as a major cause of gastrointestinal illness. Detection of *C. parvum* has advanced with the use of molecular tools; however, the difficulty of finding an oocyst in raw, finished, and waste water still exists. A virus-like double-stranded RNA particle has been identified within *C. parvum* sporozoites. The exact role of these structures is unknown, but they may affect host biology. Using viral RNA increases the chance for positive detection, as there are more than 600 structures per oocyst. In this study five techniques were used to evaluate *C. parvum* oocyst infectivity and virus presence. Water samples seeded with *C. parvum* oocysts were incubated at 25°C, 20°C, and 4°C for 8 months. Each month samples were removed and subjected to mouse and cell culture infectivity, PCR analysis for infectivity and viral particle presence, and protein analysis. While infectivity declined by more than 75% after one month at 25°C, viral particle load was similar to that of control samples at 4°C. Infectivity after two months at 20°C was similar to that at 4°C. While virus presence may not directly correlate with infectivity, subsequent viral coat protein analysis may provide evidence for immunogenicity. Viral localization will be analyzed using electron microscopy with recombinant viral capsid protein. Viral particles as evaluated by PCR over 8 months will be compared to localization and may correlate with host infection or parasite growth. The detection of parasite viral particles may be a possible means of evaluation of *Cryptosporidium* in water.

P228 DSC Efficacy of Hydrogen Peroxide for Reducing Post-harvest Fusarium Infection in Malting Barley

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Utilization of *Fusarium* infected barley for malting may lead to mycotoxin production and decreased malt quality. Hydrogen peroxide (HP) for treatment of *Fusarium* infected barley may prevent these safety and quality defects and allow use of otherwise good quality barley. Hydrogen peroxide was evaluated for effectiveness in reducing *Fusarium* infection while maintaining germinative energy in barley samples. Treatments included 0, 5, 10, and 15% concentrations with exposure times of 0, 5, 10, 15, 20, and 30 min. All treatments were done at room temperature. Treatments were repeated three times. 150 naturally Fusarium infected seeds per treatment were soaked with 50 ml of HP solution in a 100 ml conical flask. The seeds were agitated during exposure in an incubator shaker at a speed of 200 rpm. At 0% HP when agitated for 30 min, *Fusarium* infection increased by 30%. A statistically significant decrease of 79 to 95% in *Fusarium* infection occurred with 5% HP between 5 to 30 min. *Fusarium* infection was undetected at 10% and 15% HP agitated for 30 and 20 min respectively. With the exception of two treatments (10% and 15% HP agitated for 20 min) germination was not statistically significantly different from that of the control. The results suggest that hydrogen peroxide has the potential for treating *Fusarium*-infected malting barley.

P229 Effects of Cooking and Processing on the Reduction of Aflatoxin Content in Corn

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This study was performed to investigate aflatoxin reduction resulting from the cooking and processing of corn. Aflatoxin was produced by *Aspergillus parasiticus* ATCC 15517 on a type of corn imported from the United States. The aflatoxin-produced corn (AC) was pre-treated in three ways in order to reduce aflatoxin: exposure to sunlight for 7 days (SC); ultraviolet irradiation for 56 h (UC); and washing with water three times (WC). Four kinds of cooking and processing methods (boiling, steaming, baking and popping) were used to reduce aflatoxin in the AC control, SC, UC, and WC. These treatments produced corn gruel, corn cakes, corn bread and popcorn. The aflatoxin content in the

samples was determined by high performance liquid chromatography. The total aflatoxin level of the initial control (AC) was significantly decreased by sunlight and by UV ($P < 0.05$), and decreased by washing. After cooking and processing the AC, SC, UC, and WC, and averaging the total aflatoxin levels in the final products, the greatest reduction was found in the gruel, then the popcorn, then the corn cakes; the least reduction occurred in the corn bread. These results indicate that sunlight and ultraviolet energy could be effective factors in aflatoxin degradation in corn before cooking and processing. This study also indicates that boiling, steaming, baking and popping were helpful in reducing the aflatoxin level in the corn and that the most helpful factors were exposure time to heat and high temperature along with high pressure.

P230 Natural Occurrence of Aflatoxin and Fumonisin in Corn and Rice from Venezuela and Its Mycoflora

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Mycotoxins, common food contaminants produced by molds, are associated with a broad range of serious toxic effects, including carcinogenicity and other form of toxicity. The objectives of this study were to evaluate the natural occurrence of aflatoxin and fumonisin in corn and rice during the period 1998 to 2002, and its relationship with the total counts of molds and grade of colonization. The corn (450 samples) and rice (300 samples) from different region of Venezuela were collected from silos. Total mold counts in whole grains was performed using DRBC agar as culture media. The colonization of the grains was conducted disinfecting the grains with NaOCl 0.4%. The aflatoxin and fumonisin analysis were conducted using an immunoaffinity column chromatography method coupled to fluorometry. A low incidence of molds was observed in corn ($< 1 \times 10^2$–1.5×10^4 CFU/g) and rice (3.8×10^2–9.4×10^3). However, high levels of colonization was found in corn (12–96%) and rice (22–100%). 16.6 % of corn samples and 31.3% of rice were positive for aflatoxins, while 42.7% of corn samples and 22.4% of rice were positive to fumonisin. Main species of molds identified in corn were *A. flavus, F. moniliforme, P. citrinum, E. chevalieri, A. niger* while that *A. candidus, P. brevicompactum, P. variabili, A. ochraceus, C. lunata* and *C. cladosporoide* were isolated from rice. The incidence of aflatoxin and fumonisin in corn and rice suggest the hazards of these products. Also, the high grade of colonization reflects the poor agronomic practices and of storage of the grains increasing the risk of these substrates.

P231 Identification and Polymorphism of SopE in Isolates of *Salmonella enterica* — A Factor That May Contribute to the Appearance of Multiresistant Clones Associated with Cases of Food Poisoning in England and Wales

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The emergence of multiply resistant (MR) *Salmonella* (to ≤ 4 antibiotics) is a major problem to human health in many countries. An important feature of outbreaks of infection caused by MR *Salmonella enterica* has been the sequential appearance and subsequent disappearance of different clones of multiresistant strains. Such strains are primarily established in food animals (particularly bovines, but also poultry and pigs) and subsequently spread to humans through the food chain. Using PCR and sequencing, possible genetic factors which may have contributed to the establishment of multiresistant clones have been investigated. Of particular note has been the frequency of sopE in the ten most prevalent serotypes of *S. enterica*. SopE was identified in Typhimurium DTs 29, 44, 49, 204b and 204c, which have been involved in major epidemics or are precursors of epidemic strains. Presence of sopE varied in the remaining nine serotypes in England and Wales (Enteritidis, Virchow, Hadar, Newport, Infantis, Braenderup, Agona, Java and Stanley) but was more common in the top four. SopE is an effector protein that leads to actin cytoskeletal rearrangements and membrane ruffling. Nucleotide changes were detected throughout sopE and may result in altered specificity for certain signal transduction pathways, enabling 'fine-tuning' of host cell signalling and perhaps result in slight differences in invasion potential between the different serotypes. Although not identified in Typhimurium DT104, acquisition of sopE may play a key role in emergence of epidemic strains by increasing strain fitness.

P232 DSC Trends in Multiple Antibiotic Resistance of *Salmonella* Virchow

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Strains of foodborne *Salmonella* spp. with resistance to antimicrobial drugs are now spread worldwide. This study was undertaken to trace the generation of multiple antibacterial resistant *Salmonella enterica* serotype Virchow, and to determine the role of plasmidial and chromosomal mechanisms in conferring tolerance to antibiotics. Our model is *S.* Virchow, which has emerged as one of the prevalent *Salmonella* serotype in Israel. Like

S. Enteritidis, *S.* Virchow is normally associated with poultry and poultry products. However, while antibiotic resistance in *S.* Enteritidis has remained very low, *S.* Virchow strains have acquired resistance to multiple antibiotic agents. More than 85% of clinical isolates, isolated between 1997 and 2001, were resistant to at least one antibiotic, and about a third were resistant to four antibiotics or more. Significant increase was observed in the number of isolates resistant to chloramphenicol (from 13 to 43%), nalidixic acid (from 60 to 80%), and ampicillin (from 13 to 30%). High percentages of the isolates (21%) were found to be resistant to three specific drugs: trimethoprim-sulfamethoxazole, tetracycline and chloramphenicol, and 53% of those isolates were resistant to a fourth drug, streptomycin. All the resistant isolates were analyzed for the presence of Class 1 integrons and plasmids. An interesting 2.3kb plasmid was isolated from a strain that was resistant to four antibiotics, but sensitive to ampicillin. Unexpectedly, transformation of the purified plasmid to *Escherichia coli* strains resulted in ampicillin resistant colonies. Results indicate that susceptible bacteria may serve as gene reservoirs, harboring resistant genes that may function in other strains.

P233 Comparison of Several RNA Extraction Methods for the Recovery of Hepatitis A Virus from Fresh and Frozen Raspberries

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The major causes of non-bacterial gastroenteritis outbreaks around the world are due to the enteric viruses including Norwalk-like virus, Rotavirus and Hepatitis A viruses (HAV). Transmission of HAV occurs mainly through the fecal-oral route, by person-to person contact, or by ingestion of fecally contaminated foods and water. Shellfish, vegetables and berries in particular are often implicated in such outbreaks. The low virus dose and the presence of various inhibitors in this kind of foods increase the level of difficulty for the detection of the viral agents. In this study, several extraction methods used to recovered HAV RNA (strain HM-175) from fresh and frozen raspberries were compared. Amplification by RT-PCR was used for the detection of viral RNA. Fresh and frozen raspberries were spiked with various concentration of HAV and viruses were recovered by washing. Tissue culture infectious dose (TCID50%) on FRhK-4 cell monolayer was determined for each eluent. Three commercial extraction methods (Trizol Reagent, Ultraspec-3 and RNeasy), were used to isolate viral RNA from raspberry eluents. No amplification by RT-PCR was observed with the first two extraction methods. Detection by RT-PCR after RNA isolation with the RNeasy extraction kit was however very strong and the detection limit of the system was found to be at 1.0×10^2 TCID50%/ml of eluents. This extraction technique was found very effective for the removal of inhibitors present in raspberries and should be readily adaptable for isolation of other RNA viruses in berries.

P234 Survival of *Shigella sonnei* during Desiccation on Surfaces is Dependent upon Density of Inoculum and Inoculum Carrier

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Shigella sonnei populations declined at a significantly slower rate when inoculated onto strawberries at higher (10^8 CFU/berry) rather than lower (10^6 CFU/berry) levels. This observation was further investigated using glass cover slips as a model surface. Washed cells of *S. sonnei* suspended in 5% horse serum, 0.1 % peptone, and MilliQ water were inoculated (10 µl) onto cover slips at levels of 10^6 or 10^8 CFU/slip. In some cases, the inoculum was a mixture of 10^6 CFU and 10^8 cells inactivated by mild pasteurization (80°C, 5 min). Slips were held at 28 ± 1°C for 2 h and populations were enumerated over time on tryptic soy agar incubated at 37°C for 24 h. Cell populations remained constant for the first half hour for all carriers and cell densities and then began a rapid decline that corresponded with initiation of visible drying. Once visible drying was complete (approximately 40 min) populations continued a significantly slower decline. When the inoculum was 10^8 CFU/slip, a decrease of 1 log CFU/slip was observed after 2 h regardless of carrier. At 10^6 CFU/slip, decreases of 1 log CFU/slip with horse serum and peptone carriers and 3 log CFU/slip with MilliQ water were observed. Survival in MilliQ water could be improved by 2 log CFU/slip by co-inoculating 106 viable cells with 108 dead cells. These data have implications for design and interpretation of experiments that involve inoculation and drying of bacteria on surfaces, and in risk assessments that use these data.

P235 Attachment of *Shigella sonnei* Suspended in Irrigation Water to the Surfaces of Parsley and Cilantro Leaves

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Two shigellosis outbreaks in the United States have been associated with parsley and cilantro. Studies have been conducted on inactivation and growth conditions of *Shigella* on these produce; however, no data is available on the influence of the background microflora on their attachment, multiplication, and subsequent recovery. We studied

the influence of indigenous microflora on parsley and cilantro leaves and microflora introduced with typical irrigation water on the survival of *Shigella sonnei*. Cilantro and parsley leaves were spot inoculated with rifampicin-resistant *S. sonnei* suspended in irrigation or sterile water. The leaves were stored at 5 and 25°C for up to 144 h. Enumeration after washing and stomaching was performed on tryptic soy agar (TSA) and TSA supplemented with rifampicin. The indigenous microflora in the irrigation water did not influence the recovery or growth of *S. sonnei* on parsley or cilantro. No increase in numbers was observed at 5°C for *S. sonnei*, although both *Shigella* and the indigenous microflora survived the entire 144 h, with a slight increase in numbers observed for the indigenous microflora. At 25°C, an increase in numbers was observed over the 48 h incubation period. Measurable growth was greater on cilantro than parsley at 25°C. No difference was observed in attachment of *Shigella* to the leaves with time, as measured by hierarchical release on sequential washing. These results show that *S. sonnei* cells remain attached to parsley and cilantro for 144 h and washing with agitation for 4 min is not sufficient to reduce cells to undetectable levels.

P236 DSC Metabiotic Interactions of Plant Pathogenic Molds and *Salmonella* Poona on Intact and Wounded Cantaloupe Rind

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Salmonella Poona, a serotype rarely implicated in human infections, has recently caused several cantaloupe-associated outbreaks of salmonellosis in the United States and Canada. We measured changes in the pH of cantaloupe rind caused by growth of five molds commonly involved in postharvest decay and determined survival and growth characteristics of *S.* Poona co-infected with mold on and in rind tissue as affected by temperature. Intact and mechanically wounded sites on cantaloupe rinds were inoculated with a five-strain mixture of *S.* Poona and/or *Alternaria*, *Cladosporium*, *Fusarium*, *Geotrichum*, or *Penicillium*. Five inoculation schemes were used: mold only, *S.* Poona only, mold and *S.* Poona, mold then *S.* Poona 3 days later, and *S.* Poona then mold 3 days later. The pH of cantaloupe rinds inoculated with molds and stored at 20°C for 14 days was significantly higher ($P < 0.05$) than on day 0. However, only the pH of rinds inoculated with *Cladosporium* or *Geotrichum* was significantly higher ($P < 0.05$) on day 21 than on day 0, when cantaloupes were stored at 4°C. The initial population of *S.* Poona increased from 3.3 $\log_{10}$ CFU/sample (ca. 7 cm^2) of cantaloupe rind to populations as high as 9.5 $\log_{10}$ CFU/sample during storage at 20°C for up 14 days, regardless of coinoculation with molds. Populations of *S.* Poona decreased or remained constant at 4°C for up to 21 days. Results demonstrate that *S.* Poona can persist and grow on intact, wounded, and decaying cantaloupe rind at refrigerated and ambient temperatures.

P237 Effect of Irrigation Methods and Environmental Conditions on the Contamination and Survival of Enteric Microorganisms on Cantaloupe

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The purpose of this study was to compare the risk of microbial contamination of plants and soil using two methods of irrigation, subsurface drip irrigation (SDI) and furrow irrigation (FI). The cantaloupe were grown in a greenhouse containing 8 plots of each irrigation type. *Escherichia coli* ATCC 25922, *Clostridium perfringens*, and PRD1 coliphage were added to dechlorinated reclaimed domestic wastewater used for irrigation. Cantaloupe and soil samples were then collected at various times over a two week period. The microorganisms were eluted from the surface of the melons and the soil samples using a solution of 3% beef extract. Two experiments were performed to compare the effect of relative humidity on microbial survival. PRD1 and *E. coli* experienced greater reduction rates on the cantaloupe surface in dry conditions, with *E. coli* having the greatest reduction. *C. perfringens* reduction was not affected by humidity. There was no overall difference in the die-off rate of the study microorganisms in surface and subsurface soil samples. Cantaloupe contamination by PRD1 was greatest in FI plots while *E. coli* contamination was greater in the SDI plots. *C. perfringens* contamination of cantaloupe was not influenced by irrigation method. All organisms were found in greater numbers in the subsurface soil in SDI plots and in the surface soil of FI plots.

P238 DSC Effect of Electron Beam Irradiation on the Microbiological and Sensory Characteristics of Fresh-cut Cantaloupe Packed in Modified Atmosphere Packages

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The objective of this study was to determine the effect of electron beam irradiation on microbial load as well as sensory characteristics of fresh-cut cantaloupe over 21 days of storage. Equal numbers of whole cantaloupes were subjected to one of two

wash treatments, namely 200 ppm chlorine and water. Washed melons were cut into cylindrical pieces and 100 g of fruit were sealed in pre-labeled polypropylene bags. Three sets of packets were irradiated at 0.7 kGy and 1.4 kGy, respectively, and a non-irradiated set of packets served as the control. Packets were stored at 5°C and analyzed over days 0, 3, 6, 9, 13, 15, 17 and 21 for aerobic plate count and sensory characteristics. Objective sensory analysis was carried out for texture and color of individual pieces and subjective sensory analysis was carried out using a trained sensory panel. Day 0 microbial counts for the control, 0.7 kGy and 1.4 kGy were 4.0 log CFU/g, 2.0 log CFU/g and 0.8 log CFU/g, respectively, for water-washed and 2.7 log CFU/g, 0.7 log CFU/g and 0.5 log CFU/g, respectively, for chlorine-washed cantaloupes. There was a significant difference in microbial counts between wash treatments as well as doses of irradiation over 21 days. Texture was significantly different between the control and 1.4 kGy but not with 0.7 kGy. Data for objective and subjective sensory analysis were compared to evaluate the potential of electron beam irradiation as a tool for extending shelf life of fresh-cut cantaloupe.

P239 Incidence of *Listeria* spp. and *Salmonella* spp. on the Surface of Fresh Melons, Watermelons and Papayas, Using the Tecra Visual Immunoassay and Cultural Procedures for Their Detection

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In this work, the incidence of *Salmonella* spp. and *Listeria* spp. on melon (*Cucumis melo*), watermelon (*Citrullus vulgaris*) and papaya (*Carica papaya*) surfaces from fruits collected in wholesale (CEASA) and street markets in Campinas- São Paulo, Brazil, were evaluated. From the total of 120 fruits samples 42 were simultaneously analyzed by the TECRA Visual Immunoassay (TECRA-VIA) method and the modified BAM for *Salmonella* and by the Health Protection Branch, Canada, and the TECRA-VIA for *Listeria*; the remaining 78 fruit samples were analyzed only by the cultural procedures. The results showed that *Salmonella* spp. was absent in all 42 samples analyzed by both methodologies, with one false positive by TECRA-VIA. However *Listeria* spp. was detected in one sample (2.38%) of the analyzed samples, with 2 false positive and 3 false negative results by using the TECRA-VIA method. *Salmonella* spp. was also absent from 78 samples analyzed only by the modified BAM method. However *Listeria* spp. was detected in 9 (7.50%) of the analyzed samples, with *L. innocua* and *L. grayii* being isolated from watermelon, *L. ivanovii* from papaya and *L. welshimeri* from melon, without any detection of *L. monocytogenes* when using only the Health Protection Branch method. The samples collected from the street market showed a higher frequency of *Listeria* spp. when compared with the ones collected wholesale.

P240 DSC Physical and Chemical Treatments for Control of *Salmonella* on Cantaloupe Rinds

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Nalidixic acid-resistant *Salmonella* was spot-inoculated (~5.6 log CFU/cm^2) onto cantaloupes, which were then treated with tap water, chlorine dioxide, peroxyacetic acid (PA), dimethyl dicarbonate (DMDC), ozonated water, steam pasteurization, and a combination of steam/ozonated water. Excised rinds (25 cm^2) were pummeled in 50 ml DE broth, diluted in buffer and plated onto tryptic soy agar containing 50 ppm nalidixic acid. Chlorine dioxide (90 sec at 200 ppm) reduced *Salmonella* populations from 6.22 to 3.72 log CFU/cm^2, while 100 ppm chlorine dioxide (up to 90 sec) was not significantly ($P < 0.05$) better than tap water. After 45 s at 40 ppm, PA reduced the *Salmonella* population from 6.99 to 4.43 log CFU/cm^2, while longer treatment times and higher concentration (80 ppm) of PA were no more effective. Treatment of cantaloupes for 3 min with 10,000 ppm DMDC reduced the population of *Salmonella* from 5.01 log CFU/cm^2 to undetectable levels. *Salmonella* populations on cantaloupes treated with ozonated water (~1.66 ppm) were reduced by only 3.12 log CFU/cm^2 after treatment for 1 h. Further reductions after ozone treatment for up to 4 h were not significantly greater. Steam pasteurization reduced *Salmonella* populations from 5.36 to 1.70 log CFU/cm^2 after 2 min, with no increase in the internal temperature of cantaloupes. Steam treatment for 30 sec followed by ozone treatment for 20 min reduced *Salmonella* populations from 5.50 log CFU/cm^2 to undetectable levels. The observation that the steam/ozone combination effectively reduced populations suggests that additional hurdles may provide complete elimination of *Salmonella* on cantaloupes while allowing for reduced intensity of individual treatments.

P241 Fine Scale Measurement of Fruit Surface Area

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Raw or minimally processed fruits and vegetables are increasingly implicated as a source of microbial pathogens. Often, these microorganisms

are found in higher numbers on outer surfaces rather than the interior regions. Surface pathogens may penetrate to interior or edible portions through cutting or juicing, or may cross-contaminate food preparation areas and handlers. The objective of this study was to determine if the surface area of a fruit could be measured on a fine scale to determine the area available for bacterial attachment. Microscopic evaluations of antimicrobial interventions could be evaluated on the basis of changes to product surface area, in addition to viewing the presence or absence of (attached) bacteria. A new food surface area measurement technique was used to determine the relative surface area of a strawberry using different scales of measurement. Several locations on strawberries (including seeds) were viewed with a scanning confocal microscope and measured with SURFRAX®, a specialized program for determination of scale related fractal properties of measured surfaces. As the scale of measurement decreased from 10,000 μm^2 to 1 μm^2, the relative surface area increased as much as 25%. This technique may be an important way to determine the surface area of rough food surfaces and provide a determination of the area available for bacterial attachment. Furthermore, the surface area of raw produce could be evaluated after various manufacturing processes, antimicrobial interventions or storage time to quantitatively determine changes in surface area available for bacterial attachment.

P242 Microflorae of Orange Surfaces and Juice from Fruit for Processing

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Studies were conducted on citrus fruit surface and juice microflorae from oranges and grapefruit removed from various points in the process flow. Experiments were conducted at both bulk concentrate and fresh juice processors, and encompass the typical grading/cleaning/sanitation practices normally observed in citrus processing facilities. Testing included aerobic plate counts (APC), total coliforms, fecal coliforms, *E. coli* and *Salmonella.* Depending on the type of processor, fruit was removed at the following three sampling points: pre-wash and sanitation, post-wash and sanitation, and culls or pre-grading, post-grading and culls. Fruit surface APCs were generally in the range of 1000 to 10000 colony forming units (CFUs) per fruit in either the pre-wash and sanitation sampling point (fresh juice processor) or the pre-grade sampling point (bulk concentrate). In both cases, cull fruit exhibited a predictably higher APC per fruit, with numbers approaching 10 million CFU/fruit in warm weather or with over mature fruit. APCs of juice made from fruit sampled at the various points ranged from 1 to 10^0 CFU/ml for washed/sanitized fruit or graded fruit, and 1000 to 100000 CFU/ml for cull fruit. In general, both surfaces and juice of cull fruit were much more likely to be contaminated with coliforms, *E. coli* and *Salmonella* than intact, non-defective fruit. These results demonstrate the importance of cleaning/grading/sanitation of fruit for processing, as well as the necessity of proper culling for the production of wholesome citrus juice.

P243 Survival of Pathogenic and Spoilage Microorganisms in Orange Juice as Influenced by Calcium Supplements

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This study investigated the influence of calcium supplements: lactate, citrate, and triphosphate, calcium supplement mixtures (1:1): lactate-gluconate, lactate-citrate, and lactate-triphosphate, and a calcium supplement complex: citrate-malate, on the fate of pathogenic and spoilage microorganisms in orange juice. Non-fortified orange juice was supplemented with each calcium supplement or calcium supplement combination at a concentration equivalent to 10 or 30% Reference Dietary Intake (RDI). Juice pH was adjusted to 3.6 and 4.1, respectively. Each fortified juice was inoculated separately with a 3-strain mixture of *Salmonella*, a 3-strain mixture of spoilage yeasts, and 2 single strains of spoilage bacteria including *Alicyclobacillus acidoterrestris* and *Lactobacillus sakei.* Contaminated juice was stored at 4° and 10°C, respectively for 7 weeks. Juice was assayed once a week for populations of *Salmonella*, spoilage yeasts and spoilage bacteria. The results showed that *A. acidoterrestris* was inhibited in all juice at 4° and 10°C. *L. sakei* declined and eventually died off in low, as well as in high pH juice at 4° and 10°C. While inhibited at 4°C, spoilage yeasts grew in orange juice at 10°C. *Salmonella* died off in all juice at 4°C and in low pH juice at 10°C except the no-calcium controls. Cells of *Salmonella* became undetectable in high pH juice at 10°C with the exception of the control juice, juice supplemented with 30% RDI of triphosphate or citrate, and juice with 10% RDI of lactate-gluconate or lactate-triphosphate.

P244 DSC Effects of Apple Development Stages on the Internalization of *Escherichia coli* O157:H7 as Observed under Field and Laboratory Conditions

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Internalization of *Escherichia coli* in apples was investigated in orchards in Virginia, New York and Washington. Environmental conditions (sunlight, rain, temperature) were measured in each orchard. Redfree variety of apples were spray inoculated on the tree at the beginning of the growing season and

one month into the growing season with surrogate species *Escherichia coli* ATCC 25922 at 10^6 CFU/apple. Apples were harvested every other day for one week after each spray and sectioned into skin, flesh, inner core, outer core blossom end, and outer core stem end. Within 24 h after inoculation there was a 6-log reduction of *E. coli* ATCC 25922 in all samples. Red Delicious, Golden Delicious, and York apples were spray inoculated at 10^8 CFU/apple two weeks prior to anticipated harvest date, and were harvested every other day. After day one *E. coli* ATCC 25922 was <10 CFU/apple in all varieties. After three days, *E. coli* ATCC 25922 was only recoverable in approximately 40% of enrichment samples of the outer core sections. *E. coli* O157:H7 was used in an intensive laboratory study using bruised and punctured apples, with the apples examined by laser confocal microscopy. Bruising and puncturing of apples simulates post harvest injury to the fruit, which is thought to play a role in internalization. Results of this multi-state project would indicate that pathogen contamination of apples most likely occurs after harvest and that environmental conditions, sunlight, amount of rain, etc. in the orchard are important factors to decrease internalization.

P245 Modeling of *Escherichia coli* O157:H7 Inactivation by UV Irradiation and Different pHs in Apple Cider

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Foodborne illness outbreaks associated with the consumption of unpasteurized fruit juices has led to increased interest in the safety of these products. Currently, pasteurization and ultraviolet irradiation are two processing methods used in the apple cider industry. For pasteurization, it has been demonstrated that longer hold times are required for higher pH apple ciders. The objective of this study was to determine if any interactions between UV irradiation dose and pH exist for the inactivation of *E. coli* O157:H7 in apple cider. Commercially prepared apple cider was adjusted to varying pH values using malic acid or sodium hydroxide, and inoculated with *E. coli* ATCC 25922, a non-pathogenic surrogate for *E. coli* O157:H7. The various samples were exposed to varying UV doses using a commercial ultraviolet processing unit (CiderSure 3500). Bacterial populations for treated and untreated samples were enumerated using nonselective media. The results revealed that UV dose is the primary factor affecting the inactivation of *E. coli* while pH does not show a significant effect. UV exposures of 12,000 to 17,640 mW-s/cm^2 were sufficient to achieve 5-$\log_{10}$ to 7-$\log_{10}$ reduction of *E. coli* in apple cider. A generalized linear model in UV dose fitted the log reduction for the experimental range. Ultraviolet dose was the sole factor affecting inactivation of *E. coli* in apple cider. Increasing levels of UV exposure resulted in a increase in the log reduction of *E. coli* independent of the pH of the apple cider.

P246 DSC The Efficacy of Antimicrobial Treatments for the Inhibition of *Alicyclobacillus acidoterrestris* in Apple and Tomato Juices

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The objective of this study was to evaluate eight antimicrobials, i.e., sodium benzoate (SB), potassium sorbate (PS), nisin (NS), ascorbic acid (AS), cinnamic acid (CA), sodium metabisulfite (SM), lysozyme (LZ), and dimethyl dicarbonate (DMDC) for their ability to inactivate the spoilage bacterium, *Alicyclobacillus acidoterrestris* in apple and tomato juices. Shelf-stable juices were inoculated with 4 log spores/ml juice. Antimicrobials were added to result in the following concentrations: 1000, 500 and 250 ppm (SB, PS, SM); 500, 250, and 125 ppm (CA, DMDC, and AS); 125, 75 and 25 ppm (LZ); and 5, 3, and 1 IU/ml (NS). Inoculated, antimicrobial-treated juices were incubated at 42°C and sampled consecutively for 5 days, then every other day for 29 days. Juice samples were surface plated, using a spiral plater, onto Orange Serum Agar and incubated at 42°C for 48 h. The most effective antimicrobials against *A. acidoterrestris* in apple juice were: LZ (= 25 ppm), NS (= 3 IU/ml), and CA (125-250 ppm) which resulted in a 3-log or higher population reduction with no spore outgrowth observed. In tomato juice, LZ (= 25 ppm), NS (= 1 IU/ml), PS (500 ppm), and CA (= 125 ppm) were most effective, resulting in a 4-log or higher population reduction with no spore outgrowth observed. Therefore, the use of certain antimicrobials (i.e., LZ [=25 ppm], NS [= 3 IU/ml], and CA [125-250 ppm]) may provide an intervention option to prevent *A. acidoterrestris*-spoilage of both apple and tomato juices.

P247 DSC Survival of *Listeria monocytogenes* in Fruit Juices during Refrigeration and Temperature-abusive Storage

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Survival of *Listeria monocytogenes* in apple, orange, red grape, and white grape juices was evaluated. A six-strain cocktail of *L. monocytogenes* was used to inoculate (approx. 7 log CFU/ml) juices, which were stored at 4 and 10°C for up to 48 days. Inoculated red grape juice was stored

for up to 10 h only. Samples were withdrawn at appropriate intervals, neutralized with 1.0 N NaOH, serially diluted in 0.1% peptone water, and surface plated onto tryptic soy agar + 6% yeast extract (TSAYE) and Modified Oxford Agar (MOX), followed by incubation at 32°C for 48 h. When no longer detected by direct plating, samples were enriched for *L. monocytogenes* using Listeria Enrichment Broth (LEB), followed by isolation on MOX. *L. monocytogenes* remained viable in white grape, apple, and orange juices for up to 14, 22, and 48 days, respectively. In red grape juice, the microorganism was not recoverable via direct plating or enrichment at 10 h. Generally, recovery was better on TSAYE than MOX, indicating that the pathogen became acid-injured during storage. The results of this study demonstrate the ability of *L. monocytogenes* to survive in apple, orange, and white grape juices during refrigeration and abusive storage conditions. Therefore, measures to prevent or eliminate *L. monocytogenes* during fruit juice processing are necessary to ensure the safety of juice products.

P248 Simultaneous Determination of Multi-pesticide Residues in Vegetables

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Rapid and accurate methods for detecting pesticide residues in fresh food products are necessary to monitor the usage of pesticide and to provide vegetables with low or no pesticide residues for the marketplace. Our objectives were to determine the accuracy and precision of three different cleanup methods for multiresidue measurement of seven pesticides in selected vegetables (turnip greens, dried beans and cabbage). The cleanup methods for acetonitrile extraction of the vegetables included: Florisil solid phase extraction (SPE), Double SPE using C18 and amino propyl cartridges, and Direct injection of sample extract (DSI). The pesticides included ethalfluralin, bromoxynil, dimethoate, alachlor, bentazon, pendimethalin and esfenvalerate. Pesticides were measured using GC-MS with selective ion monitoring (SIM). Three of the pesticides, ethalfluralin, pendimethalin and esfenvalerate, were recovered by Florisil SPE and DSI, while dimethoate was recovered by double SPE and DSI. Alachlor, ethalfluralin, pendimethalin and esfenvalerate were recovered at high enough percentages by SPE to be measured in each vegetable. The coefficients of variation (CVs) for the four pesticides using SPE and for dimethoate using Double SPE ranged from 5.6 to 38.4%, and were of similar magnitudes as those reported by other researchers. The limits of detection of the pesticides in the turnip greens acetonitrile extract matrix ranged from 0.00075 (alachlor) to 0.19 ppm (dimethoate) and were, with the exception of dimethoate, lower than the limits of detection reported by others. Neither bromoxynil nor bentazon could be recovered consistently by any of the three methods. Therefore, Florisil SPE method is recommended as a multiresidue method for alachlor, ethalfluralin, pendimethalin and esfenvalerate pesticides. Further research is needed to find a suitable method for bromoxynil and bentazon pesticides.

P249 Reduction of Cyanide Contents of Grains, Beans and Vegetables by Thermal Treatment

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Cyanide compounds, widely distributed in many plants, inhibit cellular oxidative processes in small amounts but may be associated with chronic neurological toxicity resulting in mortal harm. The object of this research was to evaluate the effects of thermal treatment on the reduction of cyanide content in grains, beans and vegetables, and thus collect some useful information for the processing of these agricultural products. The concentration of cyanide in grains, beans and vegetables was analyzed, and blanching or steaming was applied to examine the effect on cyanide reduction. Cyanide contents in raw grains were from 0.01 in acorn to 47.88 ppm in glutinous millet and were from 0.01 to 191.89 ppm in pinto bean and soybean, respectively. When grains were cooked by soaking at 30 min followed by steaming for 7 min, the concentration was decreased about 15–50%, and the concentration in beans was decreased about 20–55%. The amounts of cyanide in vegetables were from 0.10 in Belgium to 4.59 ppm in red head lettuce. When these were blanched for 1, 3 and 5 min, the concentration was decreased with blanching time, which resulted in 40–90% of cyanide reduction. These results show that blanching and steaming with soaking could decrease the concentration of cyanide in grains, beans and vegetables. These will be used further to estimate the dietary exposure of Koreans to cyanide and to assess any potential risk associated with the ingestion of these foods.

P250 Microbial Quality of Parsley and Welsh Onion Mixture Minimally Processed Commercialized at the Supermarkets in Campinas/SP, Brazil

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The market for minimally processed vegetable food increased considerably in the last decade in

Brazil. These products are washed, peeled, cut or sliced, packed up and stored at refrigeration temperatures (8–10°C) and considered ready to eat. However little is known about microbial quality of these foods and there is a possibility of risk to the consumer's health since most of the steps such as cutting, washing and packing are made by hand, increasing the possibility of microbial contamination. Therefore, the aim of this work was to verify the efficiency of the processing on the reduction and control of the microorganisms of parsley and welsh onion mixture minimally processed, which is commonly used to season foods in Brazil. The samples were collected at four supermarkets in five different periods with an interval of two months between each sampling and submitted to microbial analysis of pathogenic *Salmonella* sp., total and fecal coliforms and total count of yeast and molds (deteriorate microorganisms). *Salmonella* was not detected. However the results showed high contamination of total coliforms (10^8 CFU), yeast and mold besides of the presence of fecal coliforms above the limit established by the Brazilian low (Resolution RDC 12/01). Probably the high microbial counting was due to the excessive food handling. Also by cutting the leaves, nutrients are liberated from the cells giving better conditions for the microbial growth, affecting the shelf life of the product. Therefore, more attention must be given by the processors during the procedure in terms of microbial control.

P251 Genetic Diversity and Antibiotic Resistance Profiling of *Salmonella* Isolated from Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas

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Fresh produce has been repeatedly implicated as a vehicle in the transmission of foodborne gastroenteritis. In an effort to assess the risk factors involved in the contamination of fresh produce with human pathogenic organisms, a total of 1,257 samples were collected from cantaloupe, oranges, and parsley in the field and after processing, and the environment (i.e., irrigation water, soil, equipment, etc.). Samples were collected twice in a season from two production farms with operating packing sheds per commodity and analyzed for the presence of *Salmonella*. A total of 25 Salmonellae were isolated. Sixteen, 6, and 3 isolates were obtained from irrigation water, packing shed equipment, and washed cantaloupe, respectively. Serotyping, pulsed-field gel electrophoresis, and repetitive sequence-based polymerase chain reaction assays were applied to all *Salmonella* isolates to evaluate the genetic diversity of the isolates and to determine if there are relationships between sources of contamination. Salmonellae isolates obtained from washed cantaloupe were most closely related to isolates obtained from equipment and irrigation water; however, DNA fingerprinting did not conclusively determine relationships between sources of contamination. In addition, all of the isolates were subjected to antimicrobial susceptibility testing using the disk diffusion method. Twenty percent (5 out of 25) of the isolates demonstrated intermediate sensitivity to streptomycin. One *Salmonella* isolate from cantaloupe demonstrated resistance to streptomycin. The other two isolates from cantaloupe were not resistant to any of the antibiotics tested. This information will be useful to the produce industry as they develop strategies to improve the safety of fresh produce.

P252 Metabiosis of Proteolytic Molds and *Salmonella* in Raw, Ripe Tomatoes

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Growth of some genera of molds in uncooked tomatoes is known to promote growth and toxin production by *Clostridium botulinum*. The objective of this study was to determine the behavior of *Salmonella* in raw, ripe tomatoes co-infected with proteolytic molds. Survival and growth of *Salmonella* as affected by co-infection of the radial pericarp tissue of sound (not chill injured) and chill-injured tomatoes was monitored. Tomatoes were inoculated with a five-serotype mixture of *Salmonella enterica* and/or *Alternaria alternata* (two strains), *Cladosporium herbarum*, and *Cladosporium cladosporioides*. Simultaneous and delayed (3 days) inoculation of tomatoes with *Salmonella* and each mold was studied. Growth of all molds in inoculated sound (not chill injured) tomatoes stored at 15 and 25°C for up to 10 days was accompanied by increased pH (up to 6.8) of pericarp tissue, which enhanced the growth of *Salmonella*. An initial *Salmonella* population of ca. 3 $\log_{10}$ CFU/g increased to 9 $\log_{10}$ CFU/g of tomato tissue co-inoculated with *A. alternata*. Growth of molds and *Salmonella* at 25°C was enhanced in tomatoes that had been chill-injured (held at 4°C for 13 days) compared to sound tomatoes. It is concluded that growth of proteolytic molds in tomatoes stored at conditions simulating those used in commercial postharvest storage and handling promotes the growth of *Salmonella* that may be an incidental contaminant. Discarding tomatoes infected by molds is important in handling and minimal processing practices designed to minimize the risk of human salmonellosis.

P253 Ionizing Radiation Sensitivity of *Listeria monocytogenes* and *L. innocua* Inoculated on Endive (*Cichorium endiva*)

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Ionizing radiation inactivates the pathogenic bacteria that can contaminate leafy green vegetables. Leaf pieces and leaf homogenate of endive (*Cichorium endiva*) were inoculated with the pathogen *Listeria monocytogenes* or *Listeria innocua*, a non-pathogenic surrogate bacterium. The radiation sensitivity of the two strains were found to be similar, although *L. innocua* was more sensitive to the type of suspending leaf preparation. During refrigerated storage following irradiation, the population of *L. monocytogenes* on inoculated endive was briefly suppressed by 0.42 kGy, a dose calibrated to achieve a 99% reduction. However, the pathogen regrew after 5 days until it exceeded the bacterial levels on the control after 19 days in storage. Treatment with 0.84 kGy, equivalent to 99.99% reduction, suppressed *L. monocytogenes* throughout the course of refrigerated storage. Doses up to 1.0 kGy had no significant effect on color of endive leaf material, whether taken from the leaf edge or the leaf midrib. The texture of leaf edge material was unaffected by doses up to 1.0 kGy, while the maximum dose tolerated by leaf midrib material was 0.8 kGy. These results show that endive leaves may be treated with doses sufficient to achieve at least a 99.99% reduction of *L. monocytogenes* with little or no impact on the product's texture or color.

P254 DSC Inactivation of Ozone Alone or Combined with Organic Acids against *Escherichia coli* O157:H7 and *Listeria monocytogenes* Inoculated into Ready-to-Use Vegetables

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This study was conducted to determine the effect of ozone and organic acids, singly or combined, on *Listeria monocytogenes* and *Escherichia coli* O157:H7 inoculated into enoki mushroom and lettuce. The spiked mushroom and lettuce were dip treated with ozone (1, 3, 5 ppm) and/or 1% organic acids for various time periods. Results showed that the treatment of 1% lactic acid for 5 min or 1% citric acid for 1.5 min against *L. monocytogenes* inoculated into enoki mushroom and lettuce exhibited the reduction of 0.71 and 1.03 log CFU/g. respectively. Similar results were observed against *E. coli* O157:H7 inoculated into enoki mushroom and lettuce. Conversely, ozone treatment up to 5 ppm did not influence decreased counts and extending the dipping to 5 min resulted in little additional decrease in counts. However, combining ozone with organic acids on enoki mushroom and lettuce significantly enhanced the reduction of *L. monocytogenes* by 1.79 and 1.84 log CFU/g and *E. coli* O157:H7 by 2.26 and 2.31 log CFU/g, respectively. Thus, the strongest synergistic effect was observed in combined ozone with citric acid samples. When 1% citric acid and 3 ppm ozone, alone or in combination treated enoki mushroom and lettuce were stored at 5°C and 15°C for designed periods, significant reductions were observed compared to control. These results showed that combined ozone and organic acids had strong antimicrobial potential to inhibit growth of *L. monocytogenes* and *E. coli* O157:H7.

P255 Cetylpyridinium Chloride and Ethanol Disinfection of Ready-to-Eat Vegetables Artificially Contaminated with *Campylobacter jejuni* and Stored at 5°C

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This study examined the synergistic effects of cetylpyridinium chloride (CPC) and ethanol (EtOH) on the survival of *Campylobacter jejuni* that was inoculated onto broccoli, celery, lettuce, mung bean sprouts, and scallions and stored at 5°C. Test portions were removed and rinsed with aqueous solutions of CPC (0.5%) for 5 min at 25°C with sonication, followed by EtOH (10%) for 1 min at 50°C. *C. jejuni* was enumerated by spiral plating on modified Charcoal-Cefoperazone-Deoxycholate Agar (CCDA). Indigenous microflorae were determined on Standard Methods (SM) agar. At initial spiking levels of 6 to 7 $\log_{10}$ CFU/g, *Campylobacter* population in the untreated produce gradually decreased throughout the storage period, showing a mean D value of 5 days for all five produce categories. No growth of *C. jejuni* in CPC/ EtOH -treated produce was observed at the lowest recovery limit (2.3 $\log_{10}$ CFU/g) of the plating method. About 1–2 $\log_{10}$ reduction (logR) in indigenous microflorae was observed. The difference in logR of 1–2 on SM agar compared to > 4-5 on CCDA suggested the limited efficacy of the disinfectants against the indigenous microflorae possibly due to the resilient and compact nature of biofilms formed on the surfaces and crevices of fresh produce. The persistent survival of *C. jejuni* up to 14 days at 5°C emphasized a need for special handling of ready-to-eat vegetables. CPC and EtOH rinses appear to be effective in controlling the viability of *Campylobacter* at simulated post-harvest contamination rates of 6–7 $\log_{10}$ CFU/g, thereby enhancing the quality of these products prior to consumption.

P256 DSC Evaluating the Efficacy of a Commercial Produce Wash on Lettuce in a Foodservice Setting

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Many microorganisms (including a number of important foodborne pathogens) can be present on raw fruits and vegetables. Since these products are frequently eaten raw, any pathogens present represent a potentially significant risk to the consumer. The objective of this study was to compare the efficacy of a commercial produce wash vs. water on reducing resident flora on lettuce during real world use by foodservice employees. Because this study was carried out in actual foodservice facilities during daily operation, we used indigenous produce microflora as pathogen surrogates. Over the course of the study, more than forty heads of lettuce were divided into thirds and each section was analyzed for total plate count (TPC) either before washing, after washing in water, or after washing in Victory™ produce wash. A slight reduction (~0.1 log CFU/g) in TPC was observed with both washing procedures, but no significant difference in reduction was detected ($P = 0.84$) between methods. When initial contamination levels were greater than or equal to 10^2 CFU/g (n = 36 samples), a significantly better reduction ($P = 0.0006$) was seen with Victory™ produce wash (1.8 log CFU/g) compared to water (0.8 log CFU/g). Our results indicate that Victory™ produce wash is effective in reducing indigenous flora on lettuce during foodservice preparation. Our results also show that care must be taken in the analysis of microbial reduction data, such that initial contamination level is considered when determining effectiveness.

P257 Ingestion of *Salmonella* Poona by a Free-living Nematode, *Caenorhabditis elegans*, and Protection against Inactivation by Sanitizers

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Free-living nematodes are known to ingest foodborne pathogens and may be vectors for preharvest contamination of fruits and vegetables. *Caenorhabditis elegans* was selected as a model to study the effectiveness of sanitizers in killing *Salmonella* Poona ingested by free-living nematodes. Aqueous suspensions of adult worms that had fed on *S.* Poona were treated with sanitizers. Treatment with free chlorine (20 μg/ml) significantly ($P = 0.05$) reduced the population of *S.* Poona compared to treating worms with water (control). There was no significant difference in the number of *S.* Poona surviving treatments with 20 to 500 μg/ml chlorine, suggesting that reductions resulted from inactivation of *S.* Poona on the surface of *C. elegans* but not cells protected by the worm cuticle after ingestion. Treatment with acidified sodium chlorite and a peroxyacetic acid-based sanitizer caused reductions of > 5.4 and > 5.3 $\log_{10}$ CFU/worm, respectively. At a concentration of 2%, acetic acid was least effective, citric acid was intermediate, and lactic acid was most effective in killing *S.* Poona ingested by *C. elegans*. Treatment of worms with up to 500 μg/ml chlorine, 1% hydrogen peroxide, 2,550 μg/ml acidified sodium chlorite, 40 μg/ml peroxyacetic acid-based sanitizer, or 2% acetic, citric, or lactic acids had no effect on the reproductive behavior. Treatments were also applied to lettuce and cantaloupe rind inoculated with *S.* Poona or *C. elegans* that had ingested *S.* Poona. Ingested *S.* Poona was protected against sanitizers applied to lettuce but not cantaloupe rind.

P258 Colonization of *Salmonella* Montevideo on Tomatoes as Affected by Relative Humidity and Storage Temperature

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A study was undertaken to determine the influence of relative humidity and storage temperature on colonization of attached cells of *Salmonella* Montevideoto on the surface of tomatoes. Red, ripe tomatoes (*Lycopersicon esculentum*) were spot-inoculated with a bacterial suspension (ca. 10^6 CFU/100ml) and stored for 90 min at 22°C under 97% relative humidity to facilitate attachment of *S.* Montevideo to the tomato surfaces. Tomatoes were then washed and stored at 22 or 30°C and relative humidities of 60, 75, 85, or 97% for up to 10 days. Periodically, tomatoes were rubbed in 0.1% (wt/vol) peptone water and washes were analyzed to enumerate populations of removed *S.* Montevideo cells. *S.* Montevideo attached to the surface of tomatoes within 90 min (3.8 $\log_{10}$ CFU/tomato). At 10 days of storage, colonization of the pathogen on tomato surfaces was greater at 30°C than at 22°C. At 30°C, the population of *S.* Montevideo increased 0.6, 1.2, 1.2, and 2.5 $\log_{10}$ CFU/tomato at 60, 75, 85, and 97% relative humidity, respectively. A similar trend was observed at 22°C, although populations were not as high. Scanning electron micrographs of washed tomato cuticles after storage revealed a well-defined biofilm containing numerous bacteria. These findings reinforce the importance of maintaining stored tomatoes at temperatures which do not support the growth of pathogenic bacteria.

P259 Survival of an Acid-resistant *Escherichia coli* Small Colony Variant in Orange Juice and Apple Cider

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A screen for mutants of *Escherichia coli* HfrH 3000 grown to log phase in LB medium and resistant to killing at pH 3.0 at 37°C was devised. Among mutants isolated were slow-growing colonies (small colony variants). One, mutant B3, has been studied in detail. Log-phase mutant cells are 3 logs more resistant to acid killing in pH 3.0 LB at 37°C than WT. The mutant was confirmed to be *E. coli* by the API 20E test, its ability to conjugate like HfrH3000 and revertant analysis. Because there are well documented cases of outbreaks of *E. coli* and *Salmonella* from orange juice (OJ) and apple cider (AC), it is of interest to determine what enotypes/phenotypes of these bacteria permit survival in these food commodities. The WT and mutant were grown to log phase in LB and diluted 1:10 (1×10^7 cells/ml) in either OJ or AC, pH 3.7–3.8 and 3.65–3.7, respectively. Three temperatures, 37, 25 and 5°C were used with both food commodities. In OJ at 2 h at 37°C, the mutant to WT survival ratio was 300, at 23 h at 25°C, 910 and in 3 days at 5°C about 5500. In apple cider the respective ratios were 7500, 2600 and 15,000. At 5°C in both OJ and AC there was no survival of the WT strain at 5 days, but the mutant was detectable even at 10 days. It will be of interest to determine if small colony variants can be isolated directly from orange juice and apple cider.

P260 Influence of Inoculation Method and Spot Inoculation Site on the Efficacy of Acidic Electrolyzed Water against *Salmonella* spp. on Lettuce

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Bacterial inoculation methods of fresh produce is a critical experimental issue concerning the assessment of the effectiveness of sanitizers. In previously reported studies, the effectiveness of different sanitizers on produce has been inconsistent. In the present work, spot and dip inoculation methods were employed to test the effectiveness of acidic electrolyzed water (AcEW) against *Salmonella* spp. Ten pieces of lettuce leaf (5 cm × 5 cm) were inoculated by each method and then immersed in 1.5 L of AcEW, sodium hypochlorite (200 ppm available chlorine) or deionized water for 1 min with agitation (150 rpm) at room temperature. The outer and inner side of the lettuce leaf was distinguished in the spot inoculation. The initial population of *Salmonella* inoculated by the dip, inner and outer spot methods was 7.72, 7.34 and 7.28 $\log_{10}$ CFU/g, respectively. Treatment with AcEW and 200 ppm chlorine equally resulted in a 1 $\log_{10}$ CFU/g reduction of *Salmonella* population by dip inoculation. With spot inoculation onto the inner side surface of the lettuce leaf, AcEW and 200 ppm chlorine reduced the number of *Salmonella* by 2.60 and 2.75 $\log_{10}$ CFU/g, respectively. With spot inoculation onto the outer side surface of the lettuce leaf, treatment with AcEW and 200 ppm chlorine resulted in 5.15 and 5.12 $\log_{10}$ CFU/g reductions, respectively, in *Salmonella*. These results indicate that a reasonable assessment of the efficacy of sanitizers on lettuce needs to take into consideration the nature of the inoculation method and inoculation site.

P261 Interaction of Foodborne Pathogens with Plant Tissue: An Active or Passive Process?

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Foodborne illnesses linked to the consumption of fresh fruits and vegetables have increased in recent years. This rise has been attributed to increased consumption of fresh produce, changes in agronomic practices, and increased importation. Limited information is available on the interaction of enteric foodborne pathogens with plant tissue. No literature is available suggesting foodborne bacteria express specific molecules to facilitate the interaction with plant tissue. Cell surface factors that may contribute to adherence include capsule and pili. Capsule may aid in adherence of a microbe to certain surfaces and pili are often implicated in adherence of the bacterial cell to inert structures as well as epithelial and intestinal cell lines. Studies in our laboratory suggest that polystyrene fluorescent microspheres (1 mm diameter) behave similar to bacteria in attachment experiments. In a series of experiments lettuce sections were immersed for 30 s in suspensions of either *E. coli* O157:H7GFP (10^7 CFU/ml) or fluorescent microspheres (10^7 beads/ml). Sections were removed, allowed to air dry and then viewed directly with a fluorescence microscope or processed to determine number of beads per cm^2 of lettuce. Based on a quantitative filtration technique the degree of adherence of beads (5.16 log bead/cm^2) to lettuce prior to rinsing was similar to that of *E. coli* O157: H7GFP (Log CFU/cm^2) to lettuce. Level of adherence after rinsing was similar for beads and *E. coli* O157: H7GFP (5.15 beads, 5.07 log/cm^2; *E. coli* O157: H7GFP, 5.08 log CFU/cm^2).

Fluorescence microscopy revealed beads localized at sites that bacteria are reported to adhere; stomata, wounds, leaf veins. These results suggest adherence of enteric foodborne pathogens to lettuce is likely a passive process and that beads can be used as a surrogate for studying the interaction of bacteria with growing plants under field conditions.

P262 Fate of Avirulent *Salmonella enterica* serovar Typhimurium on Selected Vegetables Grown in Fields Treated with Contaminated Manure Composts or Irrigation Water

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Three different types of composts, PM-5 (poultry manure compost), 338 (dairy manure compost) and NVIRO-4 (alkaline stabilized dairy manure compost), and irrigation water were inoculated with an avirulent strain of *Salmonella enterica* serovar Typhimurium at 10^7 CFU/g and 10^5 CFU/ml, respectively, to determine the persistence of Salmonellae in soils contaminated by these composts or irrigation water and also on various vegetable plants grown in these amended soils. Compost was applied to soil as a strip at a rate of 2 tons/acre (41.6 grams per square foot) before the seedlings were transplanted. A split-plot block design plan was used for each crop with five treatments (one without compost, three with each of three composts, and one without compost but applied with contaminated water) and five replicates for a total of 25 plots for each crop. *Salmonella* survived for an extended period of time, with the bacteria surviving in all soil samples for 161 days, and for 63 days on lettuce, 84 days on radishes, 166 days on parsley and 203 days on carrots. *Salmonella* survival was greatest in soil amended with poultry compost and least in soil containing alkaline-stabilized dairy manure compost. Survival profiles of *Salmonella* on plant and soil samples contaminated by irrigation water were similar to those observed when contamination occurred through compost. Hence, both contaminated compost or irrigation water can play an important role in contaminating soil and plants with *Salmonella*.

P263 Fate of *Escherichia coli* O157:H7 in Manure Compost Applied to Soil to Grow Vegetables in a Growth Chamber

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Survival of green fluorescent protein (GFP)-expressing *Escherichia coli* O157:H7 was determined in manure-amended soil used for growing selected vegetables in a controlled environment. A 36-sq. ft. Conviron plant growth chamber was used for growing green onions and carrots. A five-strain mixture of GFP-expressing *E. coli* O157:H7 was prepared and inoculated at 10^7 CFU/g into commercial dairy manure compost. The inoculated compost was mixed with Tifton sandy loam soil at a ratio of 1:100. Twenty horticultural plastic plant pots for each vegetable were filled with inoculated and fertilized soil. Three baby carrot or green onion seedlings were planted into each pot approximately 10 cm apart and placed into the chamber. Soil, plant roots, and plant surface samples in triplicate were analyzed for *E. coli* O157:H7 at weekly intervals for the first four weeks, then every 2 weeks for the remainder of the plant growth cycle (up to 3 months). For onions, the population of *E. coli* O157:H7 in soil and soil under roots steadily decreased by 3 log CFU/g over a period of 64 days, and by 2 log CFU/g on plant tissue. For carrots, *E. coli* O157:H7 populations decreased in soil by 2.3 log CFU/g during 84 days, and on plant tissues by 1.7 log CFU/g within 70 days. Results indicate that the type of plant grown is an important factor influencing the survival of *E. coli* O157:H7.

P264 A Dynamic Model for Inactivation of *Listeria monocytogenes* during Fermentation of Green Table Olives

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A dynamic model was developed to describe microbial interaction and the death of *Listeria monocytogenes* during the fermentation of green table olives. The fermentation was carried out with two different strains of starter cultures *Lactobacillus plantarum*, and supplemented with glucose or sucrose (0, 0.1, 0.3, 0.5 and 1%). The developed model was based on the following: yeasts were the only competitor of starters since they managed to attain equal levels to the latter by the end of fermentation. The other two members of initial natural flora (Enterobacteriaceae, pseudomonads) were undetectable after the first 3 days of fermentation. The decrease of *L. monocytogenes* to undetectable levels occurred rapidly after an extended initial survival period of ca. 10 days. Inactivation was independent of sugar concentration, but strongly dependent on the type of carbon source. Specifically, in olives supplemented with glucose, decline of pathogen occurred more sharply than in olives supplemented with sucrose, regardless of starter strain. However in all cases, decrease of pH below 5.0 resulted in commencement of death phase.

Moreover, lactic acid was the main metabolic product of starter cultures associated with regulation of pH, and its protonated form is known to have antimicrobial properties. Therefore, death of *Listeria monocytogenes* was modeled with differential equations that illustrated the effect of changes in pH and protonated lactic acid. Differential equations were solved numerically by Runge-Kutta method. To evaluate the model, simulation curves were generated for each set of parameters and visually tested against independent fermentation data.

P265 Reduction of *Escherichia coli* O157:H7 in Cilantro by Chlorination and Gamma Irradiation

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Cilantro is one of the most widely used fresh herbs; however, it has been found susceptible to contamination because of its physical nature and harvesting techniques. Outbreaks of foodborne illnesses have been linked to consumption of raw vegetables, including outbreaks linked to *Escherichia coli* O157:H7. Chlorination is the most common procedure to disinfect fresh produce, yet it does not effectively eliminate pathogens. Non-thermal treatments like irradiation have been shown to eliminate pathogenic bacteria in fresh-cut produce without adverse effects on quality. The objective is to determine the effectiveness of chlorination and low dose gamma irradiation for reducing levels of *Escherichia coli* O157:H7 in cilantro while maintaining product quality. Cilantro was inoculated with *Escherichia coli* O157:H7 at levels approximating 10^7 CFU/gram. Inoculated cilantro was dipped in 200-ppm chlorine solution followed by irradiation with a Cobalt 60 source at 1.0 kGy. Samples were plated on Tryptose Soy Agar containing 50-μg/mL nalidixic acid (TSAN). Survival of *E. coli* O157:H7 was counted over 13 days. Analytical sensory tests using trained panels were performed at different time intervals for a total of 14-days to detect changes in yellowing, tip burn, browning, black rot, sliminess, off-aroma, and off-flavor. Irradiation at 1.0 kGy resulted in a 4.96 log reduction in *E. coli* counts, whereas combination of irradiation and chlorination resulted in a 6.3 log reduction. Chlorination alone reduced counts by 1.03 logs. Sensory tests indicated no significant differences among attributes over time or dose. Combination treatments of chlorination and low dose irradiation can significantly reduce levels of *Escherichia coli* O157:H7 in fresh cilantro while maintaining product quality.

P266 DSC Development of Fluorescence Polarization Immunoassay for the Detection of Ochratoxin A in Korean Barley

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The objective of this study is development of fluorescence polarization immunoassay (FPIA) for the detection of Ochratoxin A in barley. Ochratoxin A (OTA) is a naturally occurring toxicant produced mostly by *Aspergillus ochraceus* and *Penicillum verrucosum*. Fluorescence polarization immunoassay (FPIA) is a homogeneous immunosaay useful for rapid and accurate detection of antibody or antigen. A homogeneous fluorescence polarization immunoassay (FPIA) was developed to measure levels of OTA in barley using a TDx analyzer in photo-check mode. Tracer was synthesized OTA-EDF (ethylenediamine fluorescein thiocarbamyl) and used monoclonal antibody of OTA-MAb-3. In result was 1 ng/mL of OTA detection limit in 60 μl sample and the recovery was in the range from 90% to 97% for spiked 10, 100, 500 ppb in barley. The cross-reactivity was below 1% for aflatoxin B1, zearalenone, patulin and T-2. The results of OTA analysis in barleys was contaminated 9 samples positive (concentration level from 3 ng/mL to 10 ng/mL). In this immunoassay, no separation step was required and the total time for an assay of 10 samples was approximately 7 min.

P267 Chemical and Irradiation Treatments in Killing *Escherichia coli* O157:H7 on Alfalfa, Radish and Mung Bean Seeds

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A study was done to compare the effectiveness of electrolyzed oxidizing water, califresh-S, 200 ppm active chlorinated water and/or sterile distilled water (500C) with or without sonication and dry heat or combination treatment to eliminate *Escherichia coli* O157:H7, on laboratory-inoculated alfalfa, radish and mung bean seeds. Treatment of mung bean seeds with electrolyzed oxidizing water along with sonication followed by rinse with sterile distilled water resulted in reductions of approximately 4.0 $\log_{10}$ CFU/g of *E. coli* O157:H7, whereas reductions of approximately 1.52 and 2.0 $\log_{10}$ CFU/g were found with radish and alfalfa seeds. The maximum of 3.70 $\log_{10}$ CFU/g reductions of mung bean seeds were achieved by treating with califresh-S and 200 ppm chlorinated water along with sonication and rinse. Combination of dry heat and hot electrolyzed oxidizing water treatment along with sonication could eliminate the population of the pathogen tested in mung been seeds but was

unable to eliminate the pathogen in radish and alfalfa seeds. Other chemical treatments used were effective in greatly reducing the population of the pathogen tested in radish and alfalfa seeds, without compromising the quality of sprouts, but these treatments did not result in the elimination of pathogens from the radish and alfalfa seeds. However, combination of dry heat and irradiation treatments was found effective in eliminating *E. coli* O157:H7 on the laboratory -inoculated alfalfa, radish and mung bean seeds and it has been seen that a dose of 2.0 kGy in combination with dry heat could eliminate the pathogen completely in alfalfa and mung bean seeds, whereas 2.5 kGy dose of irradiation is required to eliminate it completely from radish seeds. Dry heat in combination with irradiation doses up to 2.0 kGy does not unacceptably decrease the percent germination and length of alfalfa sprouts, but decreases the length of radish and mung bean sprout.

P268 Growth of *Salmonella* during Sprouting of Naturally Contaminated Alfalfa Seeds as Affected by Sprouting Conditions

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Alfalfa sprouts contaminated with *Salmonella* have been linked to a number of foodborne disease outbreaks in recent years. The source of contamination is frequently the seeds used for sprouting. Many studies have examined the growth of *Salmonella* during sprouting of contaminated seeds and shown that the pathogen proliferates rapidly and reaches high numbers during sprouting. Most of these growth studies, however, were conducted using lab-scale sprouting systems under conditions very different from those used in commercial operations. Whether similar growth kinetics will be observed during commercial sprouting operations remains to be determined. We have devised a mini-drum sprouter equipped with an automatic irrigation system similar to those used in commercial operations. The growth of *Salmonella* during sprouting of naturally contaminated alfalfa seeds in this mini-drum sprouter was compared with growth observed during sprouting in jars under conditions commonly used for home sprouting. The level of *Salmonella*, while increasing by as much as 4 logs after 48 h of sprouting in jars, remained constant during the entire sprouting period in the mini-drum sprouter. The effect of sprouting temperature and irrigation frequency on *Salmonella* growth was examined. Decreasing the irrigation frequency from every 20 min to every 2 h resulted in an approx. 2-log increase in *Salmonella* counts, and increasing the sprouting temperature from 20°C to 30°C increased the *Salmonella* counts by as much as 3 logs. Finally, the effect of chemical treatment of seeds on *Salmonella* growth was examined. *Salmonella* grew to a slightly higher level during sprouting of seeds treated with 20,000 ppm calcium hypochlorite compared to levels observed with untreated seeds.

P269 Growth and Survival of *Salmonella enterica* and Enterohemorrhagic *Escherichia coli* O157:H7 on the Model Plant *Arabidopsis thaliana*

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Salmonella enterica and *Escherichia coli* O157:H7 will contaminate fresh produce. Several epidemics related to sprouts have been traced to contaminated seed. We have developed *Arabidopsis thaliana* (thale cress) as a model system to study plant factors that support pathogen growth. Under sterile conditions and 100% humidity both pathogens grew to 10^9 CFU/gm on *A. thaliana* roots and to 5×10^6 CFU/gm on shoots. Furthermore, root inoculation leads to contaminated shoot, indicating that the pathogens are capable of moving on or within the plant. Inoculation with GFP-labeled pathogens showed invasion of the roots at lateral root junctions. Movement and, to some extent, invasion were eliminated using non-motile mutants of *S. enterica*. Inoculation of the seed before planting in soil also allowed growth of the pathogens on the foliage, though to a much lesser extent. Contamination of the foliage declined as the plants matured and was undetectable at 30 days post germination. The incidence of contamination probably dropped due to exposure of the bacteria to reduced humidity and indigenous epiphytes from soil. Nevertheless, 15% of seed-pools harvested from these plants (60 days post germination) were found to be contaminated. Furthermore, seed were not sanitized by extensive washing and chlorine treatment, indicating that bacteria reside in a protected niche on the surface of the seed or within the seed. However, seed contamination with either *S. enterica* or *E. coli* O157:H7 was reduced to undetectable levels if the original plants were co-inoculated with an epiphytic bacteria isolated from *A. thaliana*.

P270 Contamination of Prawn Flesh by *Listeria* spp. during Peeling of Cooked Prawns

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Cooked prawns represent a potential risk to public health as a source of *Listeria monocytogenes*. Prawns are usually cooked shell-on, eliminating pre-harvest contamination. Post-cooking contamination and growth of *L. monocytogenes* may subsequently occur. A study was undertaken to determine the

potential for post-cooking contamination by *Listeria* spp. to spread to the flesh. *L. monocytogenes* Scott A and *Listeria innocua* CI 94 were grown in TSB at 25°C for 18 h, washed, and resuspended in PBS to ~7 log CFU/ml. Individual cooked, shell-on black tiger prawns (*Penaeus monodon*) were immersed in one of the *Listeria* cultures and dried for 30 min. The prawns were peeled, either by hand or aseptically using a scalpel and forceps. Whole shells or flesh were homogenised and counts of *Listeria* determined by plating appropriate dilutions on PALCAM agar with incubation at 25°C for 24 h. All determinations were performed in triplicate. Both bacterial species contaminated the flesh (~4.0 log CFU per prawn) at a significantly lower level ($P < 0.05$) than the shells (~5.0 log CFU per prawn). This indicated that contamination of the flesh did occur but either before, or as a result of, peeling. No significant difference ($P > 0.05$) in *Listeria* numbers on flesh was apparent as a result of the different peeling methods, which indicated contamination probably occurred pre-peeling. Subsequent dye penetration experiments confirmed that the shells of cooked prawns were permeable to liquid and provided a poor barrier to contamination of the flesh. The importance of hygiene in controlling post-cooking contamination of prawn flesh was highlighted.

P271 Hydrated Lime Treatment of Raw Salmon Inactivates External Contamination by *Listeria innocua*

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The seafood industry is seeking methods to minimize the presence of *Listeria* species in the plants. A treatment being used on raw salmon is overnight storage in a hydrated or slaked lime solution. The objective of this study was to evaluate slaked lime as an antiListerial treatment. The surfaces of fresh, commercial, headed and gutted salmon were inoculated using an overnight culture (10^9 cells/ml) of *L. innocua* ATCC 33090. One ml was spread on each salmon and allowed to sit for one h at 25°C. The fish were placed in the treatment tote containing 0.5% hydrated lime (vs. control tote = no lime) and stored at 2°C. At three h intervals, swab samples (10 cm^2) from fish skin were serially diluted in sterile 0.1% peptone water, spread-plated on modified Oxford agar and incubated at 35°C for 24 to 48 h. The pH of the treated water, salmon skin and underlying muscle were recorded. After six h storage, a 3- to 5-log reduction of *L. innocua* was observed for salmon treated with the slaked lime whereas for the control counts were 1- to 2-logs lower. Slaked lime (0.5%) increased the water pH to 12.6. Salmon skin pH increased from pH 10.8 at three h storage to pH 12.4 by six h. Salmon muscle pH was unaffected by the lime treatment. A new treatment for destroying *Listeria* in raw salmon is overnight storage in 0.5% slaked lime. The mechanism may be due to high alkalinity at which *Listeria* species are destroyed.

P272 Use of PFGE (Pulsed Field Gel Electrophoresis) to Trace the Dissemination of *Listeria monocytogenes* in a Gravlax Salmon Processing Line

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Listeria monocytogenes is a cause of concern to food industries, mainly for those producing ready to eat products (RTE). *L. monocytogenes* can survive processing steps such as curing and cold smoking and is capable of growing under refrigeration temperatures. Its presence in fish products with a long shelflife is a challenge to the susceptible population. Gravlax (or gravad) salmon or trout are refrigerated fish products not "exposed" to listericidal processes that can harbor *L. monocytogenes* and even allow its growth. In order to monitor the dissemination of *L. monocytogenes* in a gravlax salmon processing plant, looking for "hot spots" in the plant, this study was conducted. 181 strains isolated from product (79), product contact surfaces (33) and non-product contact surfaces (44) and from food handlers (925) were submitted to PFGE according to Foodnet protocol using ApaI e AscI. The profiles were compared, combined and a dendrogram based on UPGMA cluster analysis was built. Strains were also subgrouped with *Listeria antiserum* O types 1 and 4. Six clusters were identified (A-F) with cluster A harboring 120 strains representative of the different types of samples. Cluster A is indigenous to the salmon fillets and is well adapted to the plant environment. The product was contaminated by cluster B strains at the salting step and they could be isolated from there on. Both clusters were found in the final product.

P273 Monitoring of Levels and Tracking of *Listeria monocytogenes* Strains in a Seafood Processing Environment Using Enrichment MPN and RAPD

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It is commonly acknowledged that the presence of *L. monocytogenes* within the processing environment is difficult to control. However, little information is available on the levels of this pathogen

within processing environments and the variability of strains over time. The objective of this study was to evaluate the level and strain fluctuation of *L. monocytogenes* within a seafood-processing environment. Samples were taken on a bi-monthly basis over 9 months from six environmental locations within a frozen shrimp processing line. In addition, incoming frozen shrimp, processing brine and processed product were tested. Levels of *L. monocytogenes* were determined using a 3-tube enrichment MPN. Each enrichment was streaked, and presumptive *L. monocytogenes* colonies were subjected to metabolic testing and confirmation using PCR. The detection limit for environmental and product samples was 0.03 MPN/cm^2 or g, respectively. When present, the population of *L. monocytogenes* (0.1 to 10 MPN/cm^2) was very small compared to the populations detected by standard plate counts (1×10^6 to 10^9 CFU/cm^2). Isolated *L. monocytogenes* strains were further characterized using RAPD with decamer primer UBC155 using previously optimized PCR conditions with low stringency annealing. DNA from mid-log phase cells was prepared using a commercial kit. Single-dose commercial RAPD analysis beads were used for the PCR reaction to ensure greater reproducibility. After electrophoresis, dendrogram analysis software was used to differentiate strains. Tracing the dissemination of *L. monocytogenes* through a food plant over time can help to identify sources of contamination thereby minimizing product contamination and hence the incidence of foodborne listeriosis.

P274 Tracking Viruses in the Food Chain

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Foodborne viral disease has emerged as a major public health problem in recent years, with Noroviruses (formerly Norwalk-like viruses, NLV) as the predominant causal agent. In New Zealand, NLV have been the most common cause of foodborne disease outbreaks since 1999. Among the reported outbreaks were a number of viral gastroenteritis outbreaks epidemiologically associated with commercially farmed New Zealand oysters, an outbreak of Hepatitis A virus (HAV) associated with blueberry consumption and several outbreaks where foodhandlers carried an identical NLV strain as infected, ill restaurant patrons. Similarly, the same NLV strain detected in stools from human cases was also identified in oyster flesh from the shellfish batches consumed. NLV have also been detected in imported oysters and in samples of feral and farmed bivalve shellfish from New Zealand shellfish growing areas. The development of internal viral RNA standards for NLV and HAV has improved quality control, reduced the possibility of false negative results and has allowed early identification of enzymic inhibitors present in shellfish concentrates. The use of magnetic bead concentration steps and semi-quantitative NLV and HAV real-time RT-PCR assays for virus detection has led to the development of rapid, cost effective, sensitive assays. Virological analysis of shellfish and other foods has proved to be a valuable tool in establishing the source of infection for foodborne gastroenteritis outbreaks, for identifying the health hazards presented by viral contamination of commercial and feral shellfish, and for assisting with development of improved guidelines for management of shellfish growing areas.

P275 Mitigation of Hepatitis A Virus in Shucked Oysters by Use of High Hydrostatic Pressure Treatment

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Viruses were epidemiologically linked to more than half of the 2,100 illnesses due to the consumption of raw or partially cooked shellfish in the United States from 1991 to 1998. The majority of the implicated shellfish were traced back to growing areas in approved status which were thought to have become contaminated by illegal overboard discharges of human waste or failures of proximal wastewater treatment facilities. In this study, the inactivation of hepatitis A virus (HAV) by high hydrostatic pressure was evaluated within oysters (*Crassostrea virginica*) that were allowed to accumulate the virus in a flow-through seawater system. Oysters were shucked and meats packaged in plastic pouches before pressure processing. Log_{10} reductions of HAV during a 1-min interval of high-pressure treatment were 1.72, 3.07, >3.07, and >3.07 at 350, 400, 450, and 500 MPa, respectively. Inactivation of HAV by 3 logs within whole oysters would require pressure ranges that are higher than currently used in commercial processing of shellstock oysters for reducing *Vibrio* sp. or for facilitating shucking.

P276 Prevalence of Enterovirus, NLV, and Microbial Indicators in Oysters Relocated to Gulf Coast Water Impacted by Municipal Sewage

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Sporadic outbreaks of shellfish-borne viral gastroenteritis occur primarily in the winter months. A survey conducted over eighteen months determined the prevalence of enteric viruses within shellfish that were relocated to an estuarine area directly impacted by a municipal sewage outfall.

Eighteen sample sets were collected and analyzed for enteroviruses, Norwalk-like Lorsdale-cluster viruses (NLV), fecal coliforms, *E. coli*, and male-specific coliphages. Of the 18 oyster sample analyzed, NLV and/or enterovirus sequences were detected in 78%. NLV sequences were found in 67% of the samples, while enterovirus was found in only 50%. Four oyster samples were negative for both NLV and enteroviruses. Of those four sample sets, two sets had the lowest accumulation of male-specific coliphage (< 0.6) and three sets were recorded as having the highest water temperature (> 29°C). Although shellfish-borne viral outbreaks occur primarily in the winter months, enteric viruses were detected frequently from shellfish directly impacted by municipal sewage when water temperatures were > 20°C.

P277 Survival and Persistence of Hepatitis A Virus and Norwalk-like Virus in Marinated Mussels

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Noroviruses or Norwalk-like viruses (NLV) and hepatitis A virus (HAV) are foodborne pathogens that have been associated with outbreaks of disease following consumption of raw or lightly cooked bivalve shellfish. Marinated mussels are a popular delicacy, but there is no information on whether these enteric viruses can survive the marination process. This study aimed to determine the survival and persistence of HAV and NLV in marinated mussels over time, using both molecular and cell culture methods where possible. HAV and NLV were inoculated into marinated mussels and marinade liquid, then held at 4°C for different periods up to 4 weeks. Survival of HAV was quantified by 50% tissue culture infectious dose (TCID50) per ml and correlated with RT-PCR results. The persistence of non-culturable NLVs in the mussels was determined by RT-PCR assay. Over 4 weeks, HAV survived exposure to acid marinade at pH 3.75, with a 1.7 log reduction in HAV titre observed in the marinated mussels. No reduction in RT-PCR titre was observed for either HAV or NLV in marinated mussels after 4 weeks, indicating that viral RNA was still intact following exposure to the acid marinade. Further experiments were carried out using fresh mussels infected with HAV and NLV to simulate preharvest contamination and subsequent marination. Persistence of HAV and NLV was evaluated using semi-quantitative real-time RT-PCR and viability by - TCID50 for HAV. The survival of infectious pathogenic viruses in marinated mussels constitutes a potential health risk and so is of concern to public health authorities.

P278 A Comparison of *Vibrio* Species Associated with Regional Oyster Harvest Sites

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In the United States, approximately 40% of reported *Vibrio* isolates from stool or blood specimens are associated with eating oysters harvested from one of three coastal regions: Gulf of Mexico, Pacific, and Atlantic coasts. Using data from the CDC *Vibrio* surveillance system from 1988 through 2001, we compared illness associated with oysters harvested from the three regions. While reporting *Vibrio* infections is mandatory in Gulf states, it is voluntary in all others. Oyster harvest site information was available for 436 (35%) of 1,243 persons with *Vibrio* sp. isolated from stool or blood specimens. Of these, 273 (63%) persons ate oysters harvested from the Gulf coast, 143 (33%) from the Pacific coast, and 20 (5%) from the Atlantic coast. *V. vulnificus* was isolated from 202 (71%) persons consuming Gulf coast oysters; 230 (80%) were hospitalized, and 117 (41%) died. *V. parahaemolyticus* was isolated from 134 (94%) persons eating Pacific coast oysters; 13 (9%) were hospitalized, and one (1%) died. *V. parahaemolyticus* was isolated from 15 (75%) persons eating Atlantic coast oysters; six (30%) were hospitalized and one (5%) died. Of the 139 reports that included site status, only four (3%) reported harvesting from Interstate Shellfish Sanitation Conference-prohibited sites. Gulf coast oysters were most commonly linked with *V. vulnificus* infections whereas Pacific and Atlantic coast oysters were most commonly linked with *V. parahaemolyticus* infections. Consumption of Gulf coast oysters may carry a higher risk of severe illness and death than consumption of oysters from other areas.

P279 Selectivity and Specificity of a Chromogenic Medium for Detecting *Vibrio parahaemolyticus*

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The major drawback of the widely used most probable number procedure for detecting *Vibrio parahaemolyticus* is that the use of thiosulfate-citrate-bilesalts-sucrose (TCBS) medium cannot differentiate growth of *V. parahaemolyticus* from *Vibrio vulnificus* or *Vibrio mimicus*. Recently, a chromogenic (Bio-Chrome *Vibrio*) medium was developed for differentiating *V. parahaemolyticus* from other *Vibrio* species based on the formation of purple colonies by *V. parahaemolyticus* on the medium. This study examined the selectivity and

specificity of the Bio-Chrome *Vibrio* medium (BCVM) for *V. parahaemolyticus* detection. A total of 106 bacteria including various *Vibrio* species were enriched overnight and streaked onto BCVM and TCBS plates. The plates were incubated at 37°C for 18 to 20 h and examined for bacterial growth and color development. All 36 strains of *V. parahaemolyticus* grew on BCVM, with 35 of them producing purple colonies. The other 27 *Vibrio* including *V. vulnificus*, *V. mimicus*, *V. cholerae*, *V. hollisae*, *V. alginolyticus*, and *V. furnissii* were either unable to grow or produced blue green, brown or white colonies on BCVM. Growth of *Salmonella*, *Shigella*, *Escherichia coli*, and *Yersinia* was inhibited by both BCVM and TCBS. However, *Aeromonas* was able to grow on BCVM and produced white colonies. The Bio-Chrome *Vibrio* medium is capable of differentiating *V. parahaemolyticus* from other species, including *V. vulnificus* and *V. mimicus*. It can be used together with TCBS for rapid screening of *V. parahaemolyticus*.

P280 Rapid Identification of *Vibrio vulnificus* by Real-time TaqMan PCR from Seawater

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Vibrio vulnificus is an important cause of human foodborne illness (life-threatening primary septicemia) associated with the worldwide consumption of seafood, and contaminated food and drinking water. Traditional diagnostic testing for *Vibrio* is not always reliable, because this bacterium can enter a viable but nonculturable state. Therefore, nucleic acid-based tests have emerged as a useful alternative to conventional enrichment testing. In this study, a survey was carried out to examine seawater and sediment for the presence of *V. vulnificus* at a coast in Western Korea during early and late summer using real-time TaqMan PCR and conventional PCR, bacteriological system (BioLog). Primers and probe were designed from the hemolysin/cytolysin gene sequence of *V. vulnificus* strains. *V. vulnificus* was detected by real-time TaqMan PCR, conventional PCR, and BioLog system in 53 (36.8%) and 36 (25%), and 10 (6.9%) of 144 samples, respectively. The detection method of real-time TaqMan PCR assay was better than the conventional PCR and BioLog system. Real-time TaqMan probe and primer set developed in this study can be used as a rapid screening tool for the presence of *V. vulnificus*.

P281 Use of an Acid Phosphatase Assay to Detect Deviations in Thermal Processing of Seafood

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Viral pathogens, including Hepatitis A virus (HAV) and Norwalk-like viruses (NLV), have been implicated in foodborne outbreaks associated with seafood consumption. In August 2000, imported clams were implicated in an outbreak of viral gastroenteritis in New York State. According to the manufacturer, the clams were packaged, thermally processed, labeled as "cooked," frozen, and shipped to the U.S. Upon receipt, the physical appearance and texture of the thawed clams indicated that they were raw. Microbiological analyses also demonstrated that the clams were contaminated with high fecal coliform counts, HAV and NLV. The purpose of the current study was to determine if acid phosphatase (ACP), a heat labile enzyme found in raw muscle tissue, could be assayed to determine adequate thermal processing of seafood. Raw seafood products (lobster, shrimp, oysters, crab, mussels, and quahogs) were thermally processed to an internal temperature ranging from 130 to 165°F (55 to 75°C), and acid phosphatase levels determined using the ThermaZyme ACP assay. Using the ACP curves derived from the thermal processing experiments for the seafood products, precooked seafood products were obtained and evaluated for adequate thermal processing, including the implicated clams from the 2000 outbreak. The results from this study demonstrated that the ACP assay verified adequate thermal processing of several precooked seafood products (crab, shrimp, lobster) but the implicated clams exhibited high levels of ACP, indicating they were undercooked. The information from this study may be of importance to seafood processors or regulatory agencies that are interested in identifying another method to verify thermal processing of seafood.

P282 Application of a Fluorescent Probe to the Direct Detection and Enumeration of *Escherichia coli* in Shellfish

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Escherichia coli is part of the natural flora of the intestines of animals and humans, but there are several strains capable of causing illness. These strains (ETEC, EPEC, EHEC, EIEC) have being isolated from water, beef, poultry, vegetables, milk

and dairy products, and are responsible for diseases of symptoms which may vary from a mild diarrhea to a severe hemorrhagic colitis. Infants and the elderly are more affected, and the mortality rate of EHEC on these risk groups can be as high as 50%. Although there are many methods well established for the detection of this microorganism in food, positive results can only be obtained after 3 days. Therefore, it is necessary to develop rapid methods for the detection of *E. coli*, being already available techniques as commercial kits and PCR. Using a 21-mer oligonucleotide probe described by Regnault et al. (2000), a rapid method for the direct detection of *E. coli* in shellfish, based on Fluorescent *in situ* Hybridization, was developed. The protocol was first tested against 98 *E. coli* strains isolated from food samples, and then applied to the direct detection and enumeration of this microorganism in shellfish samples. The FISH technique allowed the detection and enumeration of *E. coli* in *Crassostrea* spp., *Scrobicularia plana* and *Spisula solida*, which was confirmed by conventional microbiological techniques. Results show that this protocol can be optimised for the rapid and direct detection and enumeration of *E. coli* in food and environmental samples, allowing positive results in approximately 5 h.

P283 Histamine-related Hygienic Qualities and Bacteria Found in Popular Commercial Scombroid Fish Fillets in Taiwan

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To elucidate the histamine related hygienic qualities and bacteria of scombroid fish fillets sold in traditional retail markets, 61 samples were collected from northern and southern Taiwan. It was found that the content of volatile base nitrogen (VBN) in most samples was acceptable in middle freshness quality. The ratio of unacceptable aerobic plate count (APC) and *E. coli* was 100% and 15% in northern samples, and 100% and 20% in southern samples, respectively, compared to hygienic standards requirements. The average contents of various biogenic amines in all samples were lower than 3 mg/100g except histamine with an average content of 4.6 mg/100g in southern samples. Among southern samples, two samples contained 26.0 and 28.8 mg/100g histamine, more than the 20 mg/100g that is the allowable limit set by the Food Drug Admimistration of United States. Furthermore, 14 bacterial strains were isolated from sailfish fillets on selective medium specific for histamine-forming bacteria. These presumptive histamine-forming strains, representing genera such as *Proteus*, *Enterobacter*, *Klebsiella*, *Rahnella* and *Acinetobacter*, have been identified and found to produce histamine at levels of 20 to 2,000 ppm, after incubation at 37°C for 24 h.

P284 Monitoring of Total Volatile Basic Nitrogen, Trimethylamine Nitrogen and Biogenic Amines in Salted and Dried Chub Mackerel

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Salted and dried chub mackerel (*Scomber japonicus*) is a semipreserved fish product manufactured in the Southeast of Spain from ancient time (Villegas, 2001), and its intake could be related to the scombrotoxin syndrome. The aim of our research was to monitor the evolution of total basic volatile nitrogen (TBVN) and trimethylamine nitrogen (TMA-N), and the content of biogenic amines (putrescine, spermine, histamine, cadaverine, tyramine, phenylethylamine and tryptamine) in dried and salted chub mackerel stored at 4 and 20°C for 8 weeks. TVBN and TMA-N were determined following the methods described by Antonacopoulos and Vyncke (1988) and Dryer (1945), respectively. Biogenic amines were analyzed by HPLC as described by Yen and Hsieh (1991). TVBN and TMA-N contents in samples increased significantly during storage, with a final content of 15.28 mg/100 g and 2.42 mg/100 g, respectively, in samples stored at 20°C. Biogenic amines, tryptamine, tyramine, phenylethylamine, cadaverine and histamine, were detected during the storage at both temperatures. However, the total content of biogenic amines evaluated in the 8th week was significantly higher in samples at 20°C (420 ppm) than in samples at 4°C (300 ppm). This fact showed that bacteria with decarboxilase activity could grow during storage at both temperatures. As a conclusion and in terms of food safety, temperature should be controlled during the shelf life (6 months) of the product to avoid the formation of biogenic amines

P285 Withdrawn

P286 Baseline Risk Study of Chemical Contaminants in Ontario Farm-raised Rainbow Trout

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Despite the generally recognized health benefits of eating fish rich in omega-3 fatty acids, recent attention has focused on the safety of farm-raised fish as a food source. Chemical contaminants could

be introduced into aquaculture-raised product through veterinary therapeutic agents, contaminated fish oil-rich diets, and the environment. As part of its ongoing review and development of a comprehensive food safety program, the Ontario Ministry of Agriculture and Food conducted a baseline assessment of chemical contaminants in farm-raised trout. Samples of fresh, whole market-ready rainbow trout (*Oncorhynchus mykiss*) were collected from individual lots supplied by both land-based and cage producers in Ontario. Tissue samples were analysed for 10 veterinary drugs, 5 metals, 26 organochlorine pesticides and 7 organophosphate pesticides, as per CFIA/US EPA guidelines. Residues of 42 analytes were not detectable in any samples. Analyses showed the presence of oxytetracycline [n = 106, 1 detectable residue: 0.062 μg/g, Minimum Detection Level (MDL) 0.050 μg/gl, florfenicol (n = 126, 10 residues: range 0.1-0.3 ng/g, MDL 0.1 ng/g), sulfadimethoxine (n = 93, 3 residues: 0.056–0.096 μg/g, MDL 0.01 μg/g), mercury (n_{pooled} = 23, 1 residue: 0.07 μg/g, MDL 0.07 μg/g), selenium (n_{pooled} = 23, 23 residues: 0.2–0.3 μg/g, MDL 0.1 μg/g), and arsenic (npooled = 23, 23 residues, 0.39–0.83 μg/g, MDL 0.01 μg/g). These results indicate that levels of the tested chemicals in farmed trout are below current Health Canada MRLs. This information will be used to develop appropriate science-based risk management programs for Ontario aquaculture to ensure public health and safety, while maintaining industry competitiveness.

P287 Effects of E-Beam Irradiation on *Cryptosporidium parvum* in Eastern Oysters (*Crassostrea virginica*)

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Cryptosporidiosis caused by *Cryptosporidium parvum* can result in acute diarrhea in immunocompetent humans or life-threatening illness in immunocompromised or immunosuppressed individuals. *Cryptosporidium parvum* is a normal contaminant in many approved shellfish growing areas. The oocysts of this parasite retain their viability in contaminated oysters for extended periods post-harvest under various environmental conditions. Some customary processing operations may not eliminate *C. parvum* oocysts from oysters. The present study evaluates the effectiveness of alternative (nonthermal) processing operations to inactivate oocysts. Eastern oysters (*Crassostrea virginica*), artificially inoculated with oocysts of *C. parvum* (5.0×10^6-10.0×10^6/liter), were treated with e-beam irradiation. Doses of 1.0 and 2.0 kGy were applied to unshucked (*in vivo*) and shucked (*in vitro*) oysters. Infected oysters were irradiated on the 3rd day post-inoculation. Oocysts viability and recovery were analyzed by feeding processed material (pooled aliquots: hemolymph and gill wash; and homogenized oyster tissue) to neonatal mice. Based on mouse bioassay, *C. parvum* was detected in 30 to 40% of mice fed with oocysts irradiated at 1.0 kGy from unshucked and shucked oysters. Oocysts were not detected in mice fed oyster material treated with the dose of 2.0 kGy. This study suggests that irradiation doses equal to 2.0 kGy may serve as a commercial process to eliminate *C. parvum* in fresh oysters.

P288 Effect of Peroxyacetic Acid and Its Mixture to Eliminate Significant Foodborne Pathogens in Shrimp Processing

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The objectives of this study were to determine the alternative use of other sanitizers to substitute for chlorine based compounds in order to enhance the safety in a seafood process. Effectiveness of the two commercial sanitizers, Tsunami 100, containing peroxyacetic acid, and Vortexx ES, containing peroxyacetic and peroxyoctanoic acids, were compared for their ability to eliminate foodborne pathogens in washing shrimps. The best decontamination by using either Tsunami 100 or Vortexx ES, at concentrations of 80 to 110 ppm, for reducing *Listeria monocytogenes*, *Escherichia coli*, *Vibrio parahaemolyticus*, and *Salmonella* Typhimurium contamination on fresh shrimps, was 1 $\log_{10}$ CFU/g, at 90 ppm for 10 min. Additionally, sanitizers could be used three times within 30 min. Washing shrimps at low temperature (4°C) did not enhance effectiveness of both sanitizers compared to ambient temperature (27°C). Effectiveness of these two sanitizers in reducing pathogens were not significantly different but depended on type and load of pathogens. A storage study showed that populations of *L. monocytogenes* and *S.* Typhimurium on shrimps did not decrease during storage at -18°C for 35 days, whereas *E. coli* count decreased and was not observed after 14 days. These findings indicate that the use of two sanitizers was successful in decontamination of significant pathogens in shrimp processing and can help prevent the disease transmission in our food chain.

Technical Abstracts

T01 Evaluation of Several Modifications of an Ecometric Technique for Assessment of Media Performance

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Recovery of *Listeria monocytogenes*, *Jonesia denitrificans*, Salmonellae and *Pediococcus* sp. NRRL B-2354 across nine media was evaluated with three modifications to an ecometric method. Two modifications involved use of broth cultures (10^8–10^9 CFU/ml) of individual strains and either large (10 µL) or small (1 µL) pre-sterilized plastic loops. The third approach used pre-cultured slants and media inoculation with pre-sterilized plastic inoculating needles (10^4 CFU/needle). Absolute growth indices (AGIs) were compared. No significant differences ($P < 0.05$) were found between methods when TSAYE was used for recovery of *Listeria monocytogenes*, *J. denitrificans*, or *Pediococcus* spp. NRRL B-2354. However, use of the small loop-broth technique recovered significantly fewer *S.* Typhimurium DT104 and *S.* Senftenberg 775W than the other two techniques. Recovery of *L. monocytogenes* was excellent (AGI > 4.8) on TSAYE, PALCAM, MOX and Baird Parker, slight on modified PRAB (AGI = 0.4) and MRS agar (<0.1), but absent on remaining media (MLIA, XLD, and XLT4). Recovery of *J. denitrificans* on TSAYE and MOX was excellent but significantly greater than on PALCAM (AGI 3.0), but absent with other media tested. Recovery of *Pediococcus* sp. NRRL B-2354 was excellent on TSAYE and modified PRAB medium > Baird Parker agar > acidified MRS and absent on all other media tested. *S.* Typhimurium DT104 was recovered best on TSAYE > MLIA > XLD > XLT4 > Baird Parker > PALCAM, MOX, acidified MRS, modified PRAB, and MRS. *S.* Senftenberg 775W recovery was best on TSAYE, MLIA, XLD > XLT4 but not recovered on other media evaluated.

T02 Comparison of a Modified Plate Drop and a Solid Agar Overlay Method for Recovery of *Listeria monocytogenes* with Spread Plating and Spiral Plating Using Several Media

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Recovery of *Listeria monocytogenes* (LM) was evaluated with three quantitative techniques across three different solid media. A modified drop-plate enumeration technique (MDP) was compared with two conventional plating methods, spiral plating and spread plating, for recovery of both healthy and heat injured cells in broth and also with LM inoculated frankfurters. Media evaluated included Trypticase Soy Agar with yeast extract (TSA-YE), Modified Oxford Medium (MOX) and a novel solid agar overlay technique (TSA-YE/MOX). All experiments were performed in triplicate and each trial had three internal replicates, for a total of nine assays per data point. No significant differences ($P < 0.05$) between methods were found for recovery of LM across all three methods. No statistically significant differences ($P < 0.05$) were found for recovery of freshly grown LM inoculated onto frankfurters, after storage 4 h at 10°C, or from broth between TSA-YE and the overlay media (TSAYE/MOX). However, significantly less recovery of LM occurred on MOX medium than on TSA-YE or TSA-YE/MOX in both cases. Heat injury of LM was done at 62°C for 6 min in liquid medium. The overlay method recovered fewer heat treated LM than TSA-YE but more than MOX. The drop-plate method was shown to be capable of recovering ca. 100 cells per gram frankfurter, consistent with some Canadian and European standards.

T03 DSC Comparison and Recovery of Airborne Microorganisms in a Swine Facility Using Selective Agar and Thin Agar Layer Resuscitation Media

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Thin Agar Layer (TAL) medium was developed at Kansas State University to improve resuscitation of injured cells, and has been shown to result in higher recovery than selective media alone for cold, heat, salt, or acid injured cells. This experiment was designed to determine the effectiveness of the TAL method for the recovery of injured organisms in air. Eleven agar media were used for the experiment: Tryptic Soy agar (TSA), MacConkey Sorbitol agar (MSA), TAL-MSA, Baird-Parker agar (BP), TAL-BP, Modified Oxford agar (MOX), TAL-MOX, Xylose Lysine Sodium Desoxycholate agar (XLD), TAL-XLD, *Yersinia* Selective agar (CIN), and TAL-CIN. The TAL plates were prepared by pipetting 6 ml of a selective agar into a BBL Rodac™ plate (65 mm × 15 mm). Selective agar was allowed to solidify, then each plate was overlaid with 6 ml of TSA. Selective agar plates were prepared by pipetting 12 ml of agar into BBL Rodac™ plates and then solidifying. Samples were

taken at an indoor swine facility in 5 separate locations using a BioScience SAS air sampling instrument. For each plate, 10 L of air was sampled. Three replications of the experiment were performed. The TAL method resulted in higher total and 'Visually Typical' counts of microorganisms on all media. In addition, 175 isolates were selected randomly and identified to test the selectivity of TAL and selective media for target organisms. This data has shown that the TAL resuscitation method is a very effective and necessary procedure for the recovery of injured organisms in air.

T04 DSC Detection of Total and Pathogenic *Vibrio vulnificus* Using PCR and Oligonucleotide Microarrays

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Vibrio vulnificus is a naturally occurring estuarine microorganism found in high numbers in oysters and other shellfish. In humans, *V. vulnificus* can cause septicemia and at times death in susceptible individuals through the consumption of raw oysters. PCR amplification using oligonucleotide primers for regions of 16S rDNA and viuB was conducted to specifically identify clinical isolates of *V. vulnificus*. The results showed that 83.3% of environmental isolates had "type A" rDNA whereas 61% of the clinical isolates were "type B". On the other hand, PCR amplification of viuB followed by gene-probe hybridization exhibited positive results for 84.4% of clinical isolates and 23.3% of environmental isolates. Thus, viuB appears to be a better target for the identification of clinical strains. Next, biotin-labeled multiplexed PCR-amplified viuB and vvh gene segments were subjected to oligonucleotide microarray hybridization to detect total and clinical strains. Microarray hybridizations were carried out at 50°C and positive hybridizations detected using Tyramide Signal Amplification™ with Alexa Fluor® 546. Other shellfish-borne pathogens, *V. parahemolyticus* and *V. cholerae*, were also detected using the oligonucleotide microarray with their respective gene probes. The results exhibit that a combination of multiplex PCR and microarray hybridization permits a specific and sensitive system for detection of microbial pathogens in shellfish.

T05 Evaluation of Second Generation VIDAS® *Listeria monocytogenes* and Automated BAX® Methods for Detection of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry

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United States Department of Agriculture's Food Safety and Inspection Service published a directive for microbial sampling of ready-to-eat products to reduce *Listeria monocytogenes*. These efforts established new and intensive environmental and finished product testing guidelines for industry. Cultural methods for detecting *L. monocytogenes* can take up to 7 days to detect and identify this organism. Studies comparing method performance in detecting *L. monocytogenes* in meats and poultry provide industry with critical validated data to evaluate alternative methods that reduce time, are easy to use, and provide accurate, reliable results to ensure production of safe products. This study evaluated detection of *L. monocytogenes* by two rapid methods; bioMérieux's Second Generation VIDAS® *Listeria monocytogenes* (LMO2) and Qualicon's Automated BAX® *Listeria monocytogenes* (ABAX). Processed meat and poultry products were purchased from local grocers. Five products (hot dogs, turkey, ham, bologna, and roast beef) were evaluated by inoculating with 5 serotypes (1/2a, 1/2b, 1/2c, 3a, 4b) of *L. monocytogenes*. Twenty replicate portions for each product type were analyzed by both VIDAS LMO2 and Qualicon ABAX. Uninoculated retail samples were obtained to check for naturally occurring *L. monocytogenes*. The VIDAS method detected 63 of the 100 samples inoculated with *L. monocytogenes*, and the ABAX method detected 54 of the 100 inoculated samples. *L. monocytogenes* was not detected in the 68 uninoculated samples obtained from retail when tested by either method. Overall, VIDAS detected 9 more positive samples than ABAX. Results indicated that no statistically significant differences ($P < 0.05$) were observed between the two methods in detection.

T06 Validation of a Microwell DNA Probe Assay for Detection of *Listeria* spp. in Foods

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A new DNA hybridization assay in microwell format for detection of *Listeria* spp. in foods has been developed. This assay employs *Listeria*-specific oligonucleotide probes labeled with horseradish peroxidase and a photometrically-determined endpoint. A validation study with 15 different food commodities was conducted to compare the performance of this alternative test versus reference methods. Meats, seafood, dairy products, and fruit and vegetables comprised the categories of food tested. Food samples were inoculated at two levels and refrigerated for at least 48 h. Uninoculated (negative) control samples were included in each trial. Samples were enriched according to the procedure suggested by either the Food and Drug Administration or the U.S. Depart-

ment of Agriculture, Food Safety and Inspection Service. Samples enriched for 24 h were transferred to plates and incubated for 24 h. The surface of the plates was then swabbed and any growth present transferred to phosphate buffer solution for the performance of the DNA assay. A standard confirmation procedure was used to compare with regard to the new method to the reference method to the number of positive samples obtained. Statistical analyses of the results indicate that the proposed alternative method performs equally to cultural reference methods. The DNA assay is able to detect as low as one CFU of *Listeria* in 25-g food samples. Results can be obtained as early as 48 h after the start of sample enrichment.

T07 Nucleic Acid Sequence-based Amplification for the Rapid and Sensitive Detection of *Salmonella enterica* from Foods

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Nucleic Acid Sequence Based Amplification (NASBA) is a rapid isothermal process that targets RNA rather than DNA. RNA amplicons can be rapidly detected and confirmed using electrochemiluminescence (ECL) and adapted to multiple applications using the NucliSens® Basic Kit format. In this study, the NASBA technique was applied to the detection of *Salmonella enterica* serovar Enteritidis from representative food commodities including non-fat dry milk, chocolate, infant formula, macaroni, pepper, cake mix, chicken, beef, shrimp, chicken salad and fish. RNA was extracted using the NucliSens® Basic Kit isolation reagents from pure cultures of serovar Enteritidis, uninoculated food samples, or food samples inoculated with serovar Enteritidis that were incubated overnight in the appropriate pre-enrichment media. RNA amplification and detection was performed using previously reported primers and probes based on mRNA sequences of the dnaK gene, reagents provided by the NucliSens® Basic Kit and enzymes from various commercial sources. Serially diluted pure cultures of serovar Enteritidis gave an end-point detection limit of 10^2 CFU per NASBA-ECL reaction, similar to that found for PCR. No false positive results were obtained when enriched, uninoculated food samples were tested, indicating no significant cross-reactivity with the food matrices. All food samples inoculated with 10^1 and 10^2 CFU/25g gave positive NASBA-ECL results after pre-enrichment, except for chocolate, which had detection limits >10^2 CFU/25 g. The method was extremely robust and shows tremendous potential for the rapid and sensitive detection of low levels of *Salmonella* contamination in a wide variety of food commodities.

T08 Multiplex Nucleic Acid Sequence-based Amplification to Detect Norwalk-like Viruses (GI and GII) and Hepatitis A Virus in Food Commodities

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Human enteric viruses are increasingly recognized as important causes of foodborne disease. The most common type of viral foodborne disease is acute gastroenteritis associated with the Norwalk-like viruses (NLVs); hepatitis A infection is perhaps the most severe. The viral etiology in foodborne outbreaks can be difficult to confirm due to inadequate methods for the detection of the causative agents in food samples. In this study, a Nucleic Acid Sequence-Based Amplification (NASBA) method was developed in a multiplex format for the specific, simultaneous and rapid detection of the epidemiologically relevant human enteric viruses. Three previously reported primer sets were used in a single reaction for the amplification of different size RNA target fragments of 474, 371 and 165 nucleotides for hepatitis A virus (HAV), genogroup I (GI) and genogroup II (GII) NLVs, respectively. Amplicons were detected by agarose gel electrophoresis and confirmed by electrochemiluminescence (ECL) and northern hybridization. No significant difference in detection sensitivity was observed between the monoplex and multiplex NASBA systems. The multiplex system combined with ECL was more sensitive than conventional RT-PCR (Reverse Transcription-Polymerase Chain Reaction) (10-fold for HAV and GI but even better by 100-fold for GII). Comparable detection was obtained when representative ready-to-eat foods (deli sliced turkey and lettuce) were inoculated with various concentrations of each virus, showing the adaptability of the assay to the detection of viral contamination in foods. The multiplex NASBA system provides rapid, simultaneous and cost-effective detection of clinically relevant foodborne viruses in a single reaction tube and may be a promising alternative to RT-PCR.

T09 Rapid Enumeration of Yeast and Mold in Salad Dressings by Use of the BioSys

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Yeast and molds are common spoilage organisms of salad dressings, causing off flavors, off odors, and possible gassing in these products. Common practice in production facilities is to hold product in inventory until the results of routine testing for these organisms are complete, often up

to 5 days. An automated system for the enumeration of microorganisms, the BioSys (MicroFoss), is based on optical detection of acid or CO_2 production by use of indicators, and can provide an estimation of the level of these organisms in a salad dressing within 24 to 72 h. Acid production by yeast, or CO_2 production by mold, causes a color change in the media, which results in changes in transmittance at 585 nm. The time for detection is inversely proportional to the initial levels of organisms in the product. In this study, various types of viscous and pourable salad dressings were inoculated with several strains of yeast, and tested using yeast acid medium containing chlorphenol red as an indicator. Samples were separately inoculated with various mold strains and tested using mold medium in a vial-within-a-vial test system, with mold growth medium in an inner vial, and CO_2 indicator medium in the outer vial with thymolphalein used as the indicator. Antibacterial supplements were added to both media to inhibit bacterial growth. The inoculated samples were then incubated in the BioSys instrument at 32°C for yeast detection, or 25°C for mold detection. Differences were seen between the types of salad dressing tested, but on average 10,000 yeast per gram were detected within 15 h; 10 CFU/gram within 24 h. Detection times for mold averaged 32 h for 500,000 mold/gram and 70 h for 5.0 mold cells/gram. No interference was seen from bacterial growth in either the yeast or mold media.

T10 DSC Rapid and Specific Detection of *Penicillium expansum* by Polymerase Chain Reaction

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Postharvest spoilage of fruits results in abbreviated shelf life and significant economic losses to the fruit industry. *Pencillium expansum*, a common fruit pathogen, causes a condition known as "blue mold rot" on a variety of fruits, including apples, cherries, nectarines, and peaches. *P. expansum* is also of potential public health significance since it produces patulin, a mycotoxin known to cause toxic effects in animals. Use of moldy fruits contaminated with *P. expansum* greatly increases the risk of patulin contamination in fruit juices. Rapid and specific detection of *P. expansum* is important for ensuring both microbiological quality and safety of fruits. The traditional methods for identification of *P. expansum* are time-consuming and labor-intensive. The objective of this study was to develop and optimize a PCR for rapid and specific detection of *P. expansum*. The primers for the PCR were selected from a conserved sequence of the polygalacturonase gene of *P. expansum* published in the Genbank. The PCR amplified a 404-bp DNA product from all the ten *P. expansum* isolates tested, but not in other common foodborne *Penicillium* species and *Escherichia coli*. The PCR could also detect *P. expansum* from samples containing a mixture of DNA from *P. expansum*, *E. coli*, and other Penicillium species. Experiments to determine the sensitivity of the PCR indicated that it can detect the DNA equivalent from as low as 25 spores of *P. expansum*. The PCR could potentially be used as a rapid tool for screening fruits for the presence of *P. expansum*.

T11 DSC Nitrite-induced Injury of *Listeria monocytogenes*: Impact of Selective Versus Non-selective Recovery Procedures on Recovery from Frankfurters

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Sodium nitrite ($NaNO_2$) is used as a curing agent in frankfurters. While previous studies have documented the bacteriostatic abilities of $NaNO_2$ towards *Listeria monocytogenes*, few if any studies have been conducted that consider sublethal injury to *L. monocytogenes* by exposure to $NaNO_2$. The goals of this study were to determine if $NaNO_2$ has the ability to injure *L. monocytogenes*; to determine if nitrite-injury is reversible; and to compare the recovery of *L. monocytogenes* from frankfurters containing nitrite using *Listeria* Repair Broth (LRB) and University of Vermont Modified *Listeria* Enrichment Broth (UVM). $NaNO_2$, when used at concentrations of 100 and 200 ppm, was found to injure *L. monocytogenes*. The injury was completely reversible, or growth of uninjured *Listeria* occurred, when injury was between 98.5 and 98.7%. However, total recovery was not observed in LRB when injury exceeded 99%. UVM was unable to reverse the effects of nitrite injury in *L. monocytogenes*. Using LRB, repair of nitrite-injured *L. monocytogenes* (98% injury) began immediately and continued until total repair was achieved within 10 h at 30°C. In contrast, nitrite injured *L. monocytogenes* were unable to repair in UVM media, with injury levels remaining as high as 98% during 14 h of incubation at 30°C. With respect to time, inoculum, and meat type, LRB was found to be consistently superior to UVM in recovering *L. monocytogenes* from frankfurters. Both UVM and LRB were able to recover *L. monocytogenes* from frankfurters containing nitrites stored at 4°C until day 7, when UVM only recovered 75% of the *Listeria* population compared to 100% recovery using LRB. By the end of our study (day 28), *Listeria* was undetectable in all frankfurters using UVM, whereas LRB was able to recover 50% of the initial *Listeria* population. Nitrite-injury may be a factor influencing detection and recovery of *L. monocytogenes* from frankfurters.

T12 Pathogen Detection Using an Optical Interferometer Biosensor

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There is an increasing need for continuous, real-time monitoring of pathogens in the food-processing environment. Currently available testing methods are costly and time-consuming, requiring sample enrichment before detection or complex procedures, expensive reagents, and trained personnel. Such methods also require manual sample collection for subsequent off-site analysis. An optical interferometric biosensor, based on a planar silicon nitride waveguide, has been used for rapid and direct detection of *Salmonella* Typhimurium by use of a whole cell specific monoclonal antibody. The planar waveguide has an evanescent field that is sensitive to index of refraction changes in a layer approximately 5000 Å above the surface. The binding of antigen to antibody can be measured by combining one guided sensing beam with a reference beam in a Mach-Zender interferometer configuration. Phase changes are quantified using Fourier transform signal processing. With this approach, *Salmonella* was detected in near real time (< 30 min) by binding to antibody immobilized on a thiol-terminal silane derivatized sensor surface at concentrations in the range 10^3–10^8 cells/ml. No antibody labeling or extended incubation steps are required. Samples of poultry chiller water from a processing plant were spiked with *Salmonella* for detection within a more realistic matrix, and a prototype field-usable sensor has been tested in the laboratory. Current research includes the optimization of an assay for *Campylobacter* and development of a multi-assay sensor format. This technology promises detection sensitivity greater than that of current genetic or immunoassay techniques and a rugged design to allow its use in online process control.

T13 DSC Prevalence of *Escherichia coli* O157 among Finishing Beef Cattle Supplemented with Live Cultures of *Lactobacillus* and *Propionibacterium*

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Our objective was to determine the prevalence of *E. coli* O157 among beef finishing cattle fed a standard diet and those fed diets supplemented with direct-fed microbials (DFM). Two hundred forty steers were fed one of four treatment diets: 1) control (no added DFM); 2) NPC 747 at 10^9 CFU/steer daily plus *Propionibacterium*; 3) NPC 747 at 10^9 CFU/steer daily plus *Propionibacterium* and additional *Lactobacillus acidophilus* (NPC 750 at 10^6 CFU/steer); or 4) low dose NPC 747 at 10^6 CFU/steer daily plus *Propionibacterium*, with NPC 750 at 10^6 CFU/steer. Samples were collected from each animal and analyzed for presence of *E. coli* O157 on day 0 (fecal), 7 days before slaughter (fecal), and at slaughter (fecal and hide). *E. coli* O157 was isolated using pre-enrichment in GN-VCC broth followed by immunomagnetic separation and confirmation. Only 1.7% of cattle were positive for *E. coli* O157 at first sampling. At 7 days before slaughter and on day of shipment, prevalence of fecal positive *E. coli* O157 samples was lower in cattle receiving Treatment Diet 2 (13.3%) than in control animals (27%). For animals receiving Treatment Diets 3 and 4, prevalence was not different from animals fed the control diet. *E. coli* O157 was recovered from the hide of 13.8% of cattle fed the control diet, whereas fewer cattle receiving Treatment Diets 2 and 3 had positive samples (5.0% and 3.3%, respectively). There was no effect on animal performance. Cattle supplemented with NPC 747 had reduced *E. coli* O157 prevalence in both fecal and hide samples, indicating this treatment could effectively decrease *E. coli* O157:H7 prevalence in pre-harvest environments.

T14 DSC Factors Influencing the Recovery of Microorganisms from Surfaces Using Sterile Sampling Sponges

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The detection and enumeration of microorganisms remains an important means of assessing the hygienic status of a variety of food processing environments. Although conventional hygiene swabbing is widely used, this method is reported to recover only a small proportion of the bacteria present on a surface. Reasons for this are thought to include the limited pressure that can be applied to a swab during sampling and the relatively small area that can be sampled with each swab. In an attempt to overcome these limitations, the use of sterile sponges has been suggested as an alternative. However, information on their accuracy and sensitivity is lacking. Stainless steel coupons (81 cm^2) were inoculated with known levels of bacteria and sampled using pre-moistened, sterile sponges. Directly overlaying the sampled coupons with agar revealed that the sponges removed approximately 80% of bacteria present on a wet surface. However, only 1% of these bacteria were released from the sponge, resulting in a sampling efficiency of just 0.05%. The results also implied that this bacterial retention was not simply due to absorption of liquid. Prior to plating, squeezing the sponge to release the diluent did not improve bacterial recovery, suggesting that bacteria removed from the surface had become bound to and trapped within the matrix of the sponge. When used to sample *E. coli* and *S. aureus* the minimum detection limit of this microbiological detection method was 10^3 and 10^5 CFU/cm^2, respectively. The results of this

study strongly suggest that ineffective bacterial release is the most important contributory factor with regard to sponge sensitivity and could lead to microbial surface contamination being considerably under-estimated. The implications of these findings will be discussed in relation to the problems associated with microbiological surface sampling methods.

T15 DSC Transfer of *Listeria monocytogenes* during Commercial Slicing of Delicatessen Products

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Listeria contamination of cooked/ready-to-eat delicatessen products has become a major concern over the last five years as evidenced by at least two major outbreaks and over 80 recalls involving 130 million pounds of product. While quantitative transfer of *Listeria* in delicatessens was identified as a key informational gap in the 2001 FDA *Listeria* Risk Assessment, no published data currently exists on quantification of *Listeria* during mechanical slicing of delicatessen products. Retail blocks of Cheddar cheese (36.1% moisture, 25.5% fat) and smoked turkey breast (99% fat free) were inoculated (~10^6 CFU/cm^2) with *L. monocytogenes* Scott A and a 6-strain cocktail containing weak, medium, and strong biofilm formers. Inoculated product (3 replicates) was sliced (5 slices/replicate) at 4–7°C on a modified commercial delicatessen slicer while applying 2 and 10 lbs of force. Five product contact areas on the slicer were identified based on Glo-Germ®: the table, back plate, metal guard, blade, and product collection area. All product contact areas were sampled using a newly developed Kim-wipe® Ex-L tissue method. Using an application force of 2 lbs on turkey breast, greatest transfer was found on the metal guard (~10^3 CFU/cm^2) and blade (~10^2 CFU/cm^2) with *Listeria* transfer 10-fold higher using an application force of 10 lbs. Unlike turkey breast, Cheddar cheese transfer levels were highest on the collection area (10^2 CFU/cm^2) and blade (~10^3 CFU/cm^2) with the table yielding little or no transfer. These findings on *Listeria* transfer should help fill a critical gap in the FDA *Listeria* risk assessment.

T16 Handwashing and Gloving for Food Protection — Microbial Transfer from Contaminated Hands, Gloves, and Utensils to Food

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Both hands and gloves can be the means of transfer of foodborne and fecal microbial pathogens in a foodservice setting. The objective of this work was to critically evaluate and measure the potential for transfer of *Escherichia coli* and *Salmonella* from contaminated hands, gloves and utensils. The first phase of research studied the ability of foodborne pathogenic bacteria to remain on disposable food grade polyethylene gloves after handling food or utensils, and after alcohol sanitizer treatment. The second phase of research studied the transmissibility of bacteria from bare hands to garnish and dry food. The third and final phase of research studied the ability of bacteria to remain on gloves and utensils, and to subsequently transfer to meat. The results obtained in Phase I show that polyethylene gloves readily pick up bacteria and act as a transfer source, but the microorganisms are not easily completely removed (ranging from 20 to 80% remaining after meat or spatula handling). Phase II results show that bacteria do transfer from contaminated bare hands to dry food after minimal handling (up to 6% transfer). Phase III results demonstrate that bacteria on polyethylene gloves and on spatulas are readily transferred to ground beef orto chicken (high transfer rates ranging from 40 to 51%). In conclusion, gloves and utensils can become easily contaminated and transfer microbial foodborne pathogens to food, just like hands that have not been washed and sanitized. This work clearly demonstrates that gloves must be used properly and changed frequently to truly offer microbial protection to the customer.

T17 Air Quality Issues Associated with Hand Drying Devices in Food Processing, Food Service and Public Facility Handwash Stations

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Due to public health considerations, various indoor air quality standards have been proposed with respect to microbial counts and population types found in food production facilities, food service, healthcare, and office workplaces. Tools and equipment used in these facilities can often influence microbial counts and population types, with pathogenic species of greatest concern. Both RCS Biotest and SAS Super 100 samplers were used to obtain air samples from these types of facilities. A great deal of variation was seen in air quality from handwash station and toilet areas, with overall counts from 10^2 to over 10^4 CFU/m^3 seen. In 15 of 50 toilet areas tested, bacteria of the *Enterobacteriaceae* group were found in the air, presumably a result of toilet aerosols. Air quality is seen to be influenced by the presence or absence of hot air dryers vs. cloth or paper towels. A trend toward consistently higher aerobic plate counts, yeast and mold as well as *Staphylococcus* spp. were found in facilities with hot air dryers. From 3 different seafood processing facilities (2 in the US and

one in the UK) high microbial counts were found associated with hot air dryer usage. *Staphylococcus* spp. were frequently seen being expelled from hot air dryer air streams, coming from users' hands and being recycled through units when running. Significant bacteria and mold contamination of units were sometimes seen leading to microbial levels in air beyond suggested guidelines for food processing or workplace environments.

T18 DSC An Examination of Food Safety Risk Management Behavioral Trends of Ontario Greenhouse Vegetable Growers

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In 1998 the Food Safety Network (FSN) at the University of Guelph and the Ontario Greenhouse Vegetable Growers began the implementation of a proactive, on-farm food safety program. This initiative was designed to reduce the occurrence of microbial contamination of greenhouse vegetables by working with the 220 members of the grower group directly. The HACCP-based program focuses on the grower/packer/shipper area of the farm-to-fork food production chain. Since inception, a FSN researcher has acted as an extension liaison to program participants. Through on-site visits, frequent informal dialogue, a monthly newsletter and daily food safety information summary sheets, emerging food safety information is communicated to program participants, with the goal of reducing complacency toward food safety issues on the farm. This research reports trends discovered by measuring program compliance in addition to changes in knowledge, attitudes and practices of participants, as well as excising lessons learned through the implementation of an on-farm food safety program. Trends based on categories such as farm capital size and water source type (well vs. municipal supply) have been shown to impact the adoption and success of the program by participants. A triangulation of methods was used to establish and confirm trends including surveys, stakeholder interviews and microbial data from produce and water samples. The information gathered through this research provides a model of trends for other similar program implementation teams to follow.

T19 Assessing the Cost of Microbiological Failures to Food Manufacturers and the Primary Reasons for Product Contamination

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The sensitive commercial and financial implications of reporting product rejections for microbiological reasons has resulted in a lack of data examining the cost and key reasons for rejection. Possible causes may be cross contamination coupled with poor validation and verification within food management systems. This study evaluates the causes and extent of failures in companies producing ready-to-eat manufactured foods. Thirty-five of the highest volume products manufactured by 10 SME companies were studied. The microbiological records for the previous twelve months production was analyzed and the percentage failure rates were established. An average 1.1% of products failed to achieve 1 or more microbiological criteria specified by the company. The results were further analysed to identify trends in production cycles and other variables, including staffing levels. Seasonality in the production cycle was an important factor in failure rates of some companies. Ninety-two percent of technical staff working within the companies studied were unaware of the products most likely to fail. Only 35% of the technical staff interviewed were able to identify key contamination points within the production process and the results suggest that insufficient use was made of trend analysis techniques for the tracking of microbiological quality. A case study of one manufacturer with high seasonal rejection rates (associated costs in excess of $50,000) is presented, analysing reasons for failure, costs incurred and types of corrective actions that could have been implemented. The results are discussed within the overall economics of food safety management.

T20 Development of Information Resources to Assist Small Businesses in Hazard Identification

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To reduce the incidence of foodborne illnesses, the application of hazard and risk based quality management systems is essential. Small companies tend to have a poor understanding of such systems, leading to limited adoption of HACCP in some industry sectors. Provision of information resources has been identified as a strategy to assist small and microbusinesses in hazard identification. The aim of this project is to assess the current levels of understanding of hazards and risks, determine the format for information presentation and finally provide the required information in paper and electronic media. A questionnaire survey was made of 850 small and medium size enterprises (SMEs) and microbusinesses in the UK, covering a wide range of product types. The aim was to determine the industry sector and processes carried out, whether the company operated hazard based quality management and the knowledge of the technical manager (or equivalent)

regarding the associated hazards and risks. The questionnaires were followed up by visits to the manufacturing plant to observe the processes and the operatives to determine their level of understanding of the tasks they were performing. Preliminary results show that the majority of respondents stated that they did operate hazard based quality management. The ability of the respondents to correctly define a hazard or risk or identify different types of hazard was, however, poor. Further analysis of results will identify any correlation between variables such as company size, susceptibility of product to contamination by pathogens and the knowledge of the personnel within the business.

T21 Review of the Use of Scientific Criteria and Performance Standards for Safe Food

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An ad hoc committee and two subcommittees appointed by the Food and Nutrition Board, Institute of Medicine, and the Board on Agriculture and Natural Resources, Div. of Earth and Life Sciences, National Academy of Sciences, reviewed the scientific basis of criteria, including microbiological criteria, and performance standards, established by the regulatory agencies for meat and poultry, seafood, produce and related products (including fruit juices), and dairy products. The study originated in a request by Congress and was sponsored by the USDA and the FDA. The committee and subcommittees met during the period February to October 2002 and issued a report in March 2003. In this report, the committee evaluated the general framework currently used by the regulatory agencies to establish criteria applicable in processing of the selected food groups, and made recommendations for improvement. This evaluation covered the historical background of criteria and performance standards for food safety in the United States; the current foodborne disease surveillance system and its future needs; the need for food safety assurance from farm to table; the role of criteria within a HACCP system; and the tools (including risk assessment, statistical approaches, food safety objectives, and economics) available for establishing science-based food safety criteria. The committee also adopted or developed definitions for use in food safety regulations. The subcommittees, in turn, examined specific criteria, including performance standards, currently in place for each food group studied and made recommendations for improvement. Research needs were identified. The main findings and recommendations of the committee and subcommittees are described and discussed.

T22 Improving Urgent Public Health Information Dissemination in California:The Food Safety Notification System

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Traditionally, the primary route of urgent public health information distribution is through press releases issued by public health agencies. Although press releases are an important and necessary tool, experience has repeatedly shown this tool can be a slow and sometimes unreliable method of communication. A more direct mode of communication, one that relies less on the media's interpretation and re-distribution of the information, is needed to reach wholesalers, retailers, and consumers to prevent additional cases of illness. The California Department of Health Service, Food and Drug Branch (DHS-FDB) has implemented a new communication system called the Food Safety Notification System (FSNS). This system allows DHS-FDB to quickly disseminate information regarding food safety events directly to those who can act to prevent additional exposures. FSNS is a web-based system that allows rapid transmission of thousands of messages in multiple formats to individuals. FSNS increases the effectiveness of communication by allowing DHS-FDB to transmit urgent food safety messages, such as recalls of contaminated food, virtually instantaneously though e-mail, fax, voice mail, and pager. FDB has successfully used this communication tool with over 2,000 participants for approximately one year. During this time, FSNS has increased the effectiveness of communication between DHS-FDB and California food industry groups. The results are extremely encouraging and participants have found the system to be useful and user friendly. FDB will continue to use this communication system as a fast and reliable method to disseminate information to California food industries.

T23 DSC Development and Evaluation of an Educational Resource to Engage Senior High School Students in Dialogue Regarding Genetically Engineered Food

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In trying to understand opposition to genetically engineered (GE) foods, studies focused on high school students found that they were aware of, but had little knowledge of, genetic engineering. This paper outlines the needs assessment, development and evaluation of a printed resource (& video). The needs assessment, involving student and teacher focus groups (n=27), identified

preferred activities, information sources, topics, and characteristics along with the possibility of delivering the resource via CD-ROM. It was concluded that CD-ROM delivery was not feasible due to lack of computers. Thus, a printed resource was developed to demonstrate the connection between science, society and the environment through a series of modules ranging from basic genetics to food production to societal issues surrounding GE foods. The evaluation involved 207 senior family studies students from 4 volunteer schools (urban and rural) across Ontario in a pretest, posttest quasi-experimental design. It was hypothesized that the resource would increase students' knowledge and positively affect their attitudes towards GE foods. Gender and background (urban or rural) effects were also examined. Intact classrooms were matched within individual schools, with the same teacher instructing both the control and experimental groups. Lack of random treatment assignment did not allow between-school comparison; however, similarities were observed across the different settings. Knowledge and attitudes were assessed with a pretested, panel reviewed survey consisting of open-ended and Likert-type items (reliability assessed with test-retest method using control data). Program satisfaction was assessed through an open-ended teacher survey. The resource increased knowledge and affected student attitudes.

T24 DSC Spot the Mistake: What Television Cooking Shows Teach Viewers

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Consumers receive information on food preparation from a variety of sources. A study conducted by the Canadian Food Inspection Agency in 1998 demonstrated that Canadians rely on television as one of these primary sources. This research reports on an examination and categorization of messages that food and cooking programs provide to viewers about preparing food safely. From June 23 to 30, 2002, television food and cooking programs were recorded and reviewed, using a defined list of food safety infractions based on criteria established by Food Safety Network researchers. Most surveyed programs were shown on the Canadian Food Network, a specialty cable channel. Twenty-five individual programs were reviewed, representing a total of 48 different episodes. Twenty-eight per cent of the programs viewed were produced in Canada, with the remainder produced in the U.S. or U.K. Based on 28.5 h of detailed content analysis, a total of 384 poor food-handling incidents occurred, which can be approximated as an incident every 5 min of viewing time. Common food safety errors observed included: a lack of hand washing, cross-contamination and time-temperature violations. Television food and cooking programs provide an opportunity to promote safe food handling; however, these findings suggest that food safety practices on television cooking broadcasts could be improved.

T25 The Bactericidal Use of Ozone in the Treatment of Fresh Strawberries

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Consumers and retailers are demanding foods from manufacturers that are safer and have a longer shelf life but are also natural, fresher and produced using fewer additives. These sometimes conflicting requirements can pose problems for manufacturers of high-risk confectionery products, e.g. fresh fruit custard tarts. This type of production requires the addition of fresh fruit, e.g. strawberries which may be of variable microbial quality, to the surface of high risk products. The use of chlorinated water or similar treatments may increase the risk of mould growth, and adversely affect the product organoleptically. There are therefore no validated control measures to ensure the microbiological quality of fresh fruit. Ozone is a proven biocide, although little information is available on its effect on fruit. One study used very low levels of ozone (0.35 ppm) to treat strawberries for up to three days but this adversely affected the organoleptic properties and had little antimicrobial effect. The present study used much higher levels of ozone at 10, 50 and 100 ppm for up to a maximum of 8 h. The effect of this on the aerobic colony count and on coliforms is reported. The reduction in overall microbial numbers due to the ozone varied from 0.5 to greater than 4 log with 100 ppm being the most effective. Ozone at 50 ppm for 8 h reduced the number of coliforms by 1.5 log values. The organoleptic quality of the strawberries was not adversely affected. Ozone treatment of fresh fruit has potential as a control measure within the production of fresh fruit confectionery products.

T26 Comparison of Inoculation Methods to Determine the Efficacy of Chlorine Dioxide Gas and Chlorinated Water Treatments to Reduce *Escherichia coli* O157:H7 on Strawberries

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It is challenging to interpret efficacy data of antimicrobials using different cell inoculation procedures. Our objectives were to: a) compare spot- and dip-inoculation for efficacy of chlorine dioxide (ClO_2) gas and chlorinated water treatments to reduce *E. coli* O157:H7 on strawberries, and,

b) visualize *E. coli* O157:H7 cells using cryo-scanning electronic microscopy (CSEM). Strawberries were spot-inoculated or dip-inoculated with 7 log CFU/strawberry *E. coli* O157:H7, air-dried for 2 h, stored for 1 or 3 days at 4°C, and then treated with 0.4, 0.8, or 1.2 mg/L ClO_2 gas for 30 min at 22°C and 90–95% relative humidity, or treated with 200 ppm chlorinated water for 10 min. Bacterial populations were examined using membrane-transferring plating with tryptic soy agar and sorbital MacConkey agar. Spot-inoculation showed higher bacterial recovery than dip-inoculation and the difference increased with storage time. Inoculation procedures, storage conditions, and time affected the efficacy of sanitation treatments. After 0.8 mg/l ClO_2 gas treatment, bacterial log reductions/sample on spot- or dip-inoculated strawberries were 5.2 ± 0.4 or 4.5 ± 0.8 for 2 h-dried samples, 5.0 ± 0.5 or 4.0 ± 0.4 for 1 day-stored samples, and 3.4 ± 0.2 or 2.4 ± 0.4 for 3 day-stored samples. After 200 ppm chlorinated water treatment, differences between log reductions/sample with spot-inoculation and dip-inoculation were 1.1, 0.4, 0.6, and 1 for the samples immediately inoculated, 2 h-dried, 1-day stored, and 3-day stored, respectively. CSEM studies suggested that distribution of bacteria on strawberry surfaces was different with use of spot- and dip-inoculation methods and that bacteria on intact flesh surfaces could be more easily washed off than those on seed surfaces.

T27 Elimination of Molds on Dried Fruits and Nuts by Electron Beam Irradiation

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Some of the fungi known to infest dried fruits and nuts are known mycotoxin producers. Considering that dried fruits and nuts are normally consumed raw, the presence of fungi on such products at the retail level can be a potential health risk. Thus, there is a need to develop food preservation technologies that can eliminate or reduce fungal infestation of dried fruits and nuts. Our objective was to evaluate electron beam (E-beam) technology to eliminate fungi on dried fruits and nuts. Samples of walnuts, dates, raisins, and figs were obtained from retail sources and sub-samples analyzed for the presence and levels of fungi using 3M Yeast and Mold Count (YM) plates and Rose Bengal Agar. Triplicate samples were placed in plastic bags and exposed to E-beam irradiation doses of 0.5, 1.0, 2.0, 2.5, and 3.0 kGy. Six out of the 11 retail samples (55%) were positive for fungi and the levels in these samples ranged between 10^2 and 10^3 CFU/gm of sample. The D-10 values (kGy) for walnuts ranged between 1.2 and 1.6, while for dates it was between 1.0 and 1.2. The D-10 value for raisins ranged between 0.6 and 1.1 kGy. The lack of correlation between initial mold concentration and the D-10 values suggests relative differences in irradiation resistance among the fungal species present on these commodities.

T28 UV Disinfection of Juices: Challenges of Microbiological Validation of Flow-through Reactors

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To validate UV disinfection of juices, critical product/process parameters that affect inactivation efficiency of continuous treatment must be known. Optical properties of the juices, flow pattern and design of the UV reactor are the major factors affecting UV disinfection in media with high absorptive properties. In order to overcome the interference of absorbance and turbidity of fresh juices, thin film and turbulent flow UV reactors were employed. The objectives were to study the effects of absorbance, suspended solids in apple juice/cider on microbial destruction; and to validate the effectiveness of laminar and turbulent flow UV reactors in achieving a 5-log reduction of *E. coli*. *E. coli* K12 (ATCC 25253) was chosen as a target microorganism for biodosimetry. Malate buffer and caramel of 0.13 to 0.6% were used to simulate the effects of pH, Brix and color/absorbance of juice on inactivation rates. Effectiveness of UV inactivation in juices and models was compared in a thin film reactor CiderSure, turbulent flow "Aquionics" and annular thin film "Ultradynamics". The absorbed UV dose for 1-log inactivation of *E. coli* K12 in the liquids studied was determined based on averaged UV intensity and measured residence time in an annular reactor. Within UV reactors, the absorbance consistently affected the efficacy of UV light inactivation. Factors such as Brix and pH had no significant effect. The flow rates and mixing conditions also affected microbial inactivation: the higher the flow rates the higher the UV inactivation rates in the turbulent flow reactor. Regression equations were developed for predicting inactivation rate based on absorbance for laminar and turbulent flow reactors.

T29 Infiltration and Survival of *Escherichia coli* ATCC 25922 on Apples under Orchard Conditions

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This study was designed to understand the mode of infiltration and survival of *Escherichia coli* on apples under orchard conditions. This is important for the development of intervention steps to enhance microbiological safety of fresh produce.

A non-pathogenic *E. coli* ATCC 25922 culture was used to inoculate fertilized flowers on apple trees in April of 2001. Also, mature apples on trees were inoculated with the same strain in mid August. Whole and dissected samples of the inoculated fruit, collected in June and mid August through October (bi-weekly sampling), were analyzed for the presence of *E. coli*. Data indicated that this organism is able to survive under orchard conditions, both on apple surfaces and within the core. All *E. coli* cells isolated from fruit samples after mid-September were injured cells. These results document the *E. coli* hazard in apples from orchards located near areas of animal production where exposure to contaminated dust or irrigation water can occur and where *E. coli* may become internalized within fruit so that the bacteria cannot be inactivated or be removed by washing.

T30 Antilisterial Activity in Cut Iceberg Lettuce Extracts

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Modified atmospheres and the application of mild heat treatments delay wound-associated phenolic metabolism and the onset of browning in fresh-cut iceberg lettuce (*Lactuca sativa* L.). Growth of *Listeria monocytogenes* in packaged lettuce is known to be enhanced by such treatments. The purpose of this work is to clarify the role of wound-associated reactions in the ecology of the species. Aqueous extracts were prepared from fresh lettuce shreds and from shreds stored aerobically for three days at 10°C with and without prior treatment at 47°C for three min. The extracts were inoculated with *L. monocytogenes* (10^4 CFU/mL) and were incubated for up to 7 days at 25°C under constant agitation. Changes in cell populations were monitored by plating onto Tryptic Soy agar. Extracts prepared from fresh lettuce shreds and from shreds stored after heat treatment supported growth of the species. In contrast, extensive cell death was observed in extracts prepared from untreated lettuce stored for three days. These results indicate that antilisterial factors were formed in wounded iceberg lettuce tissues during storage. Chemical analysis yielded mean total phenolic contents of 10.5 mg/mL (expressed as chlorogenic acid) in fresh tissue extracts, 3 mg/mL in extracts prepared from untreated shreds stored aerobically for three days and 7 mg/mL in extracts prepared from stored, heat treated lettuce. Hence the cumulative effect of phenolic compounds was not responsible for the effect and it was hypothesized that antilisterial activity was due to the synthesis of specific, unidentified wound-associated compounds.

T31 Organically Grown Lettuce: Hygienic Quality and Risk of Transfer of Pathogenic Bacteria

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In May 2001, a four-year project was started in Norway to investigate the bacteriological quality of organically grown lettuce and the risks of using untreated manure as a fertilizer. The project comprises four parts where two seasons of field trials of growing of Iceberg lettuce fertilized with different fertilizers in 2001 and 2002 have recently been finished. The main objective of the field trial was to compare the bacteriological quality after fertilizing with non-organic fertilizer, composted manure, firm manure and slurry. Samples of soil prior to and after fertilizing, manure and lettuce were analysed for total aerobic counts, thermotolerant coliform bacteria/*E. coli*, *E. coli* O157, *Salmonella* spp. and *Listeria monocytogenes*. In 2001, none of the pathogens were detected and there were no difference in the numbers of *E. coli* in the lettuce at harvesting. In 2002, *E. coli* O157:H7 (stx_2+, eae+) were isolated from firm manure, slurry and manure-fertilized soil one week after spreading of the manure. *E. coli* O157:H7 were isolated from manure at one later occasion, but were not detected in lettuce or at later samplings of the soil. This random finding of *E. coli* O157:H7 was very surprising as the occurrence of *E. coli* O157:H7 in the ruminant reservoir in Norway is low. The remaining parts of the project include a composting trial to investigate the possibilities of using composting as a mean to reduce the presence of potential pathogens, a growth trial where artificially contaminated manure is to be used for the growing of lettuce and a storage study.

T32 A Survey to Determine Field and Packing House Hygiene Practices in New York

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People can contaminate fresh fruits and vegetables with human pathogens and several US produce-associated outbreaks have resulted from this source of contamination. In the summer of 2002, an oral survey of workers who harvest and pack fresh fruits and vegetables was conducted in New York State by visiting farms, migrant worker camps, clinics, and other venues frequented by workers. The objective of this study was to determine worker understanding of personal hygiene practices, including proper handwashing and toilet use, and to assess the current status of sanitary facilities on farms and in packing houses in the state. A total of 689 surveys were collected between

June and September 2002, and were coded and analyzed. Workers from 14 countries participated in the survey, with 86.5% being male and 13.5% female, and the largest percentage (37%) being between 15 to 24 years of age. The survey was completed in Spanish by 87% of the workers; 4.8% were completed in Haitian Creole, while 7.8% were completed in English. When field workers were asked if toilets are available in the field within 1/4 mile of their work, 71.5% responded always or most of the time. About 83% said they used these facilities. Almost 75% said that handwashing facilities are also available in the field and 6% said that they used them. The information gathered in this survey is being used to develop farm worker education and training materials for the National Good Agricultural Practices (GAPS) Program.

T33 DSC A Field Study of the Microbiological Quality of Fresh Produce

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Despite numerous health benefits, fresh produce has been associated with several high profile foodborne disease outbreaks. In response to new federal guidelines to minimize food safety hazards in fresh fruits and vegetables, we initiated a project to determine the specific production and processing practices associated with microbial contamination of produce. We report the levels of bacterial indicator organisms and the prevalence of select pathogens in fresh produce samples collected from the southwestern U.S. A total of 433 produce samples, consisting of leafy greens and cantaloupe, were collected throughout production and processing, followed by enumerative tests for total aerobic bacteria (APC), total coliforms, total *Enterococcus*, and *E. coli*. The samples were also analyzed for the presence of *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7. For all leafy greens and herbs, median indicator levels ranged from 10^2 to 10^3 CFU/g (coliforms and *Enterococcus*); from 10^5 to 10^6 CFU/g (APC); and <10 CFU/g (*E. coli*). Indicator levels remained consistent throughout processing for most of these products except cilantro, which showed an increase in total coliforms. For cantaloupe, microbial levels significantly increased from field throughout processing, with ranges of 10^6 to 10^7 CFU/g (APC); 10^2 to 10^4 CFU/g (coliforms); 10^3 to 10^6 CFU/g (*Enterococus*); and <10 to >10^2 CFU/g (*E. coli*). The prevalence of pathogens for all samples was 0%, 0%, and 0.7% (3/433) for *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella*, respectively. Microbiological data from these samples, along with corresponding survey data on farm and shed operations, can be used to evaluate and refine guidelines to minimize food safety hazards in fresh produce.

T34 Preliminary Evaluation of *Citrobacter* spp. as a Surrogate for *Salmonella* in Controlled Release Field Studies

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The development of recommended management practices for prevention and reduction of foodborne contamination during pre-harvest production of produce is limited by the predominant absence of pathogens. Pathogen occurrence in production environments is rare and unpredictable. In surveys of the microflora of several fruits and vegetables produced, *Citrobacter* spp. was found to be relatively common regionally and seasonally. In pathogen inoculations on produce, *Citrobacter* spp. was routinely present at the terminal phase of selective recovery of several *Salmonella* serotype strains. *Citrobacter* spp. co-purified with *Salmonella* spp. in commercial immuno-magnetic separation protocols. In a series of comparative growth, stress and recovery studies on produce, *C. fruendii* Rif 3 was determined to be a candidate surrogate for *Salmonella*. Preliminary evaluation of the environmental survival of *C. fruendii* Rif 3 isolate was tested in non-commercial field trials of lettuce. Sprays of *C. fruendii* Rif 3 resulted in detectable populations up to 12 days post-application only if the initial concentration was at least 7.0 log CFU/ml (7.6 log CFU per plant). After drying, the mean population of *C. fruendii* Rif 3 was 4.3 log CFU/plant, which decreased to 2.3 log CFU/plant (daytime 26C and solar UV measured at 95 to 137 mol m -2 sec-1). Aerosols of C. *fruendii* were detectable at least 50 m from the source. *C. fruendii* was not recovered from non-treated Romaine lettuce at this 50 m sampling site 24 h post-application. *C. freundii* Rif 3 appears to be a suitable surrogate to test environmental persistence, dispersal, and pre-harvest mitigation strategies.

T35 Pre-symptomatic Infection of Asparagus by *Pectobacterium carotovora* subsp. *carotovora* Increases Wound Co-colonization by *Escherichia coli* O157:H7 and *Salmonella* Serotypes

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Asparagus is produced in many countries and marketed globally. Although most consumption is preceded by cooking, uncooked or lightly steamed spears are popular. Hydrocooling of fresh asparagus at shipping point and secondary hydrocooling at import distribution facilities is a common practice.

Adequate disinfection of re-circulated cooling water has not been uniformly implemented. Postharvest delays to cooling and inadequate disinfection can result in the multiplication of the soft-rot pathogen *Pectobacterium carotovora* subsp. *carotovora* at permissive temperatures. A mixture of strains of *E. coli* O157:H7 or a mixture of five serotype strains of *Salmonella* were co-inoculated at equal population densities (4.0 log CFU/wound) into 1 mm wound sites. Inoculations were made at four positions from the base to 5 mm below the apical bracts. Spears were incubated at 15°C and greater than 95% RH for 72 h. All wound sites had negligible visible decay, except for the inoculation nearest the apical tip in which water-soaking was becoming apparent. The *E. coli* O157:H7 and *Salmonella* spp. increased 2 log cycles and 4 log cycles in population in the presence of *P. carotovora* at the basal portion and tip portion of the spears, respectively. *Salmonella* serotype differentiation was performed by ERIC PCR from randomly replicated single-colony selections from enumeration media. Cumulatively, ninety single colonies were subjected to DNA typing. *Salmonella* serotype Montevideo was the predominant recovered isolate with or without *P. carotovora* co-inoculation. *Salmonella* Agona was absent at 72 h. Prevention of contamination, timely cooling and proper postharvest disinfection during cooling are recommended for minimizing food safety risk.

T36 The Use of Gradient Plates to Study the Combined Effect of Temperature, pH and NaCl Concentration on the Growth of *Monascus ruber* van Tieghem, an Ascomycetes Fungus Isolated from Green Table Olives

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The effect of temperature, pH and sodium chloride concentration on the growth of the ascomycetes fungus *Monascus ruber* van Tieghem was studied using the gradient plate technique. Gradients of NaCl (3 to 9%, w/v) at right angles to pH (2 to 6.8) were prepared for the plates, which were incubated at 25, 30 and 35°C. Fungal growth, expressed in optical density units, was recorded by image analysis and graphically presented in the form of three-dimensional grids. Results obtained from the plates indicated that the fungus was salt and acid tolerant, and able to grow at NaCl concentrations up to 9% (w/v) and at low pH values from 2.2 to 3.7, depending on incubation temperature. The inhibitory effect of NaCl increased as the pH decreased progressively at 25 and 30°C but not at 35°C. Growth was better at 30 and 25°C as judged by the larger extent of the plates covered by mycelium compared with 35°C where no growth was observed at pH below 3.7. Differentiation between vegetative (imperfect stage) and reproductive (perfect stage) growth was evident on all plates, providing useful information about the effect of environmental conditions on the form of fungal growth. Overall, it is suggested that the fungus, which is the main spoilage microorganism during storage of table olives, cannot be inhibited by any combination of pH and NaCl within the limits of the brine environment, so further processing is required to ensure product stability in the market.

T37 Microbiological Risks of Handling Raw Meat in the Domestic Environment

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The aim of this research was to gain a deeper understanding of the problems and risks associated with the handling of raw meats in the domestic kitchen. This was done initially through qualitative and quantitative surveys considering the public awareness of the risks of handling raw meat products. The surveys also considered kitchen practices and meal preparation scenarios. The survey highlighted areas where the public understood good hygienic practice, but also some key areas in which their understanding was poor. Following this, members of the public were asked to prepare various meals in a trial kitchen, their procedures were observed and recorded, and microbiological tests were carried out in various parts of the kitchen before and after the meal had been prepared. Results indicated areas in which microbiological contamination could build up during meal preparation; some of these areas were not those that would be routinely cleaned in normal domestic cleaning regimes. Using results from surveys and meal preparation, laboratory research was done considering the ability of meats to hold and distribute microbiological contamination around a domestic environment. The overall outcome of this work highlights that while some information about microbiological risks of food preparation in the home is understood, there is a high degree of misunderstanding by the general public of the risks of handling raw foods in their own kitchens, and correct intervention measures to take to reduce the risks of pathogen contamination in the domestic environment.

T38 Development of a Systems-based Approach to Food Safety

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Epidemiology, laboratory science, and environmental health are important components of food

safety. Epidemiologic and laboratory findings identify microbes and provide insight into the possible direct causal association between an exposure and an adverse health outcome. However, information on environmental health factors that precede and contribute to the direct cause is often lacking. For example, a foodborne disease outbreak investigation may reveal that people became ill from *Salmonella* after eating a salad. However, often there is little information regarding how and why the salad became contaminated with *Salmonella.* This information can be obtained by using a systems-based approach to conduct the investigation, which involves all three fields of science: epidemiology, laboratory science, and environmental health. A systems-based approach is founded on systems theory, which states that to fully understand the whole, one must understand not only the individual elements, but also the interrelations among the elements that make up the whole. Using a systems-based approach will enable public health professionals to identify the agent and the vehicle of the disease, as well as better identify and quantify environmental antecedents that contribute to foodborne disease outbreaks. This approach has the potential to identify areas in which perceptions, behaviors, and practices of food workers need to be changed, which may ultimately lower the risk of developing a foodborne illness.

T39 Prevalence of High Risk Egg Handling Practices in Restaurants: An EHS-Net Survey

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In 1999, *Salmonella* Enteritidis (SE) was the second most common *Salmonella* serotype reported to the Centers for Disease Control and Prevention, accounting for more than 16% of reported isolates and approximately 200,000 cases of foodborne illness. During the past 2 decades, shell eggs have been the most commonly implicated vehicle in SE outbreaks. To determine the prevalence of "high risk" egg handling practices (e.g., pooling eggs, improper holding, inadequate cooking), information from 153 restaurants was collected through interviews regarding eight egg dishes. Final cook temperatures of three egg dishes and the internal temperature of one refrigerated shell egg in each restaurant were observed and recorded. Pooling of shell eggs was reported in 96 of 153 (62.8%) restaurants. Thirty-nine of 152 (25.7%) managers reported storing eggs at room temperature and seven (4.6%) stored eggs on ice or water baths prior to cooking. Seventy-five of 136 (55.2%) managers reported sanitizing utensils used to prepare eggs at least once in 4 h. Generally eggs were cooked from 72.0°C – 82.9°C, which is above the recommended temperature. Of 139 refrigerated shell eggs, 126 (90.7%) had internal temperatures measured below the recommended 7.0°C. Various high-risk food-handling practices were reported. Although these practices may lead to contamination or proliferation of SE in eggs, most final cook temperatures observed would reduce the risk of SE transmission. In conclusion, further surveillance of food-handling practices is needed. Emphasis on post-cooking practices, such as hand contact with cooked egg dishes, may provide insight in investigating SE outbreaks in restaurants.

T40 A Review of Operational Elements of Retail Food Protection Programs Across States

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Research conducted by the Centers for Disease Control and Prevention (CDC) indicates that eating outside the home is a risk factor for developing a foodborne illness. The Environmental Health Specialists Network (EHS-Net), a collaborative project among CDC, U.S. Food and Drug Administration, and eight states (California, Colorado, Connecticut, Georgia, Minnesota, New York, Oregon, and Tennessee), is conducting research on food safety in restaurants. One aspect of this research focuses on the structure of local food programs. In the fall of 2001, a questionnaire was sent to one environmental health specialist involved with the food program in each county in the EHS-Net catchment area. Of the 414 counties in the catchment area, 312 reported data, resulting in a response rate of 75%. Questions focused on program jurisdiction, restaurant manager certification, adoption of FDA Model Food Code provisions, use of electronic databases, and definitions of food service establishments. Results of the project indicate that the food programs within the EHS-Net catchment area vary greatly. There is no consistency in definitions of food service establishments, jurisdiction, use of electronic databases, or adoption of FDA Model Food Code provisions across states or within states. The differences in food programs make it difficult to conduct research on food program policies and practices. Research in this field would benefit from a nationwide set of programmatic standards or guidelines for food protection programs and sufficient funding for basic resources needed to implement and sustain these guidelines.

T41 Review of Studies on Food Worker Food Handling

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The majority of foodborne illness outbreaks reported through the Centers for Disease Control and Prevention (CDC) surveillance system originated in food service establishments, and research indicates that food handler behaviors are significant contributors to foodborne illness. Researchers have been increasingly studying food worker behavior, but an overview of existing data has been lacking. Thus, we conducted a review of studies on determinants of food workers' behavior, including food safety knowledge, attitudes, and practices. An extensive search of the literature was conducted to locate studies on food workers' food safety knowledge, attitudes, self-reported practices, observed practices and other determinants of food safety behavior; we then reviewed these studies. We used the following methods to identify articles included in the review: electronic searches of computerized databases, reviews of reference lists from relevant articles and reports, and browser searches of the World Wide Web. The majority of studies (40%) in food worker food safety have been conducted since 1999; the most frequent methods of data collection were surveys (46%) and observation (54%); and food safety knowledge and observed practices were assessed most frequently (57% and 50%), while attitudes were assessed least frequently (20%). In addition, the review revealed a discrepancy between knowledge and behavior – even when food workers possess knowledge of safe food handling practices, they do not always handle food safely. The review also indicated that few studies have examined determinants of food safety behavior other than knowledge, attitudes, and practices, such as barriers to implementation and perceptions of risk.

T42 Restaurant Workers' and Managers' Perceptions of Facilitators and Barriers to Safe Food Handling

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Research has indicated that eating outside the home is a risk factor for foodborne illness and that the behaviors of food workers contribute significantly to foodborne illness. These findings suggest that improving workers' food handling behavior is crucial to reducing foodborne illness. However, successful behavior modification requires an understanding of the factors underlying food safety behavior, including the facilitators of and barriers to safe food handling. Thus, the Environmental Health Specialists-Network (EHS-Net), a collaborative project of the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), and eight states, has spearheaded a focus group study on facilitators and barriers to safe food handling. This presentation will outline results from these focus groups, conducted with 112 restaurant workers and managers from randomly selected restaurants in the EHS-Net states. Restaurant workers' and managers' self-reported facilitators and barriers to handling food safely will be summarized. Specific food handling topics addressed include cooking, holding, cooling, reheating, handwashing, and cross-contamination. Participants' responses to questions concerning how issues such as food safety training, working while ill, and language barriers affect food safety will also be summarized, and disparities between workers' and managers' responses will be discussed. The information presented will contribute to our understanding of food handling practices in restaurants and improve our ability to develop effective food safety behavioral interventions.

T43 A Cooperative Approach to Retail Food Safety

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Both the retail and food service industries and regulatory jurisdictions have a responsibility for ensuring the safety of food at retail. This presentation will discuss some of the activities FDA has undertaken to assist industry and regulators to meet this challenge through the control of foodborne illness risk factors. The Centers for Disease Control and Prevention have identified these five broad categories of risk factors that most often contribute to foodborne illness at retail: 1. Food from Unsafe Sources; 2. Inadequate Cooking; 3. Improper Holding Temperatures; 4. Contaminated Equipment; and 5. Poor Personal Hygiene. FDA is encouraging industry to implement food safety management systems that focus on establishing active managerial control over these risk factors. Having active managerial control includes having procedures in place for controlling identified foodborne illness risk factors. "Managing Food Safety: A Guide for the Voluntary Use of HACCP Principles for Operators of Food Service and Retail Establishments" has been developed to assist industry in achieving active managerial control. At the same time, FDA is working with federal, state, local, and tribal regulatory jurisidictions in an effort to establish uniformity across their food safety programs. Adoption and implementation of the Food Code has historically been the keystone in achieving that uniformity. A missing piece, however, has been an agreed upon national standard or foundation for regulatory programs that administer the Food Code. The National Retail Food Regulatory Program Standards were formulated by FDA as a way to identify

program strengths and weaknesses and to develop strategies and action plans to address these issues.

T44 The Effect of Inaccurate Risk Assessment in HACCP Programs on Manufacturers Operational Performance

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The EU Hygiene Directive of 1993 (E93/43/EEC) and 1995 UK General Food Hygiene Regs effectively incorporated 5 principles of HACCP and draft EU Regs for 2002 may require 7 principles as defined by Codex. However, at present manufacturers develop HACCP programs on an individual basis. This study analyzed the methods and principles adopted by SME companies in the UK with particular emphasis on critical control point development and the method of risk assessment. A semi-structured interview of the technical representatives for the 12 partner companies was used to establish an understanding of the company's expertise on HACCP and the principles that each adopted. Only 16% of the companies named specific bacterial risks during their risk assessment exercise with the remainder merely highlighting the risks as "bacterial" when applying the Codex decision tree process for CCP production. Over 60% of the companies had more than double the number of CCPs which would have been established through the Codex decision tree process. A random audit of CCP "process control checks" was also undertaken to establish whether a link existed between the number of CCPs and the efficiency of CCP monitoring. This study also analysed the training programmes and techniques employed by the partner companies and how the effects of the training are evaluated. Financial implications of inefficient HACCP programs were also analysed and the findings will be discussed.

T45 DSC Sensitivity of *Escherichia coli* O157:H7 to Industrial Alkaline Cleaners and Subsequent Exposure to Heat

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The effects of seven alkaline cleaners, 0.05 M NaOH, 0.05 M KOH, and 0.05% peptone (control) on viability of wild-type (EDL 933) and rpoS-deficient (FRIK 816-3) strains of *Escherichia coli* O157:H7 in logarithmic and stationary phases of growth were determined. Cells were treated at 4 or 23°C for 2, 10 or 30 min. Four cleaners (# 2, 4, 6, and 7) containing hypochlorite and < 10, 11, 10, and 11% NaOH and/or KOH (pH 11.2 – 11.7), respectively, were significantly ($P < 0.05$) more effective in reducing populations compared to treatment with 0.05% peptone or two cleaners, # 3 and 5, which contained < 10% KOH and sodium metasilicate (pH 11.4) or ethylene glycol monobutyl ether (pH 10.4), respectively. Treatment with 0.05 M KOH or NaOH (pH 12.2) was not as effective as industrial cleaners in killing *E. coli* O157:H7. Lethality to both strains was similar, indicating that the rpoS gene may not play an immediate role in protecting cells exposed to cleaners and/or highly alkaline environments. Reduction in populations of stationary phase and logarithmic growth phase cells was also similar, indicating that bactericidal activity of cleaners was sufficient to overcome resistance presented by cells at different physiological ages. Stationary phase cells of strain EDL 933 treated at 4°C in 0.05% peptone, cleaner 5, or cleaner 7 for 2 min had higher D55°C values than cells treated at 23°C. This indicates that composition of alkaline cleaners, pH, and temperature at which cells are treated can affect viability and subsequent heat resistance.

T46 DSC Antibiotic Susceptibility and Cross Contamination of Enteric Bacteria Isolated from Feedlot Cattle and Their Carcasses

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The prevalence, potential for cross-contamination, and antimicrobial drug susceptibility patterns of *Salmonella* and commensal enteric bacteria in feedlot cattle and their carcasses was determined. A total of 60 animals were shipped to a commercial abattoir in three shipments. Bacterial isolates were collected from these animals immediately before shipping, at the abattoir after exsanguination, after hide removal and in the cooler. Samples were cultured for *Salmonella*, non-type specific *E. coli*, and enterococci. *Salmonella* was identified in 33.9% (n=20) of fecal samples and on 37.3% (n=22) of hides prior to shipment. At the abattoir, the proportion of hides from which *Salmonella* was isolated increased ($P < 0.01$) to 84.2% (n=48) indicating that cross-contamination of the hides occurred during shipping. Generic *E. coli* was recovered from 40.4% of preevisceration carcass samples, while *Salmonella* was recovered from 8.3% and enterococci from 58.3% of the samples. No *Salmonella* or generic *E. coli* were recovered from hotbox carcass samples. However, enterococci were recovered from 8.6% of the hotbox carcass samples. Isolates were also tested for antimicrobial drug susceptibility. Of the *E. coli* isolates processed, 25% were resistant to tetracycline, 8.3% to cephalothin and kanamycin, and 4.2% to streptomycin. Multiple drug resistance was identified in 4.2% of isolates. Analysis of these isolates by PFGE indicates a between-animal fecal to hide contamination that occurs

during transportation and similarity between bacteria isolated from carcasses and the isolates originally found on the hides. These results suggest a link between bacterial isolates found in the feedlot environment and those found on the carcasses at the plant.

T47 Determining the Prevalence of *Escherichia coli* O157 in Cattle and Beef from the Feedlot to the Cooler

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Studies have shown that prevalence of *Escherichia coli* O157 contaminated cattle entering the slaughtering floor may range from 10 to >70%. The objective of this study was to determine the effect of the prevalence of *E. coli* O157 in fecal samples from feedlot pen floors on prevalence of *E. coli* O157 on carcasses at various points in the slaughtering process. Feedlot-floor fecal samples were collected within three days before slaughter. During processing at the slaughter facility, additional samples were collected from the hide and colon, and from the carcasses before and after evisceration and after final decontamination. Of 15 lots sampled, 87% had a (at least one) positive feedlot-floor fecal sample, 54% had a positive hide sample, 80% had a positive colon/fecal sample and 47% had a positive sample pre-evisceration; however, only 6% of lots had a positive sample post-evisceration or after final intervention. Of the total samples tested (n = 1,328), 24.7, 14.7, 27.6, 10.0, 1.4 and 0.3% of feedlot-floor, hide, colon, pre-evisceration, post-evisceration and final intervention samples, respectively, were positive for *E. coli* O157. Pens with greater than 20% positive feedlot-floor fecal samples had 22.5% positive hides, 46.3% positive colons, and 12.5%, 2.5% and 0.6% positive carcasses at pre-evisceration, at post-evisceration and after final intervention, respectively. Conversely, feedlot-floor samples which contained less than 20% positive fecal samples were associated with 5.7% positive hides, 7.1% positive colons, and 7.1%, 0% and 0% positive carcasses at pre-evisceration, at post evisceration and after final intervention, respectively. Data from this study should be useful for risk assessment and/or identifying mitigation strategies to minimize prevalence of *E. coli* O157 on fresh beef.

T48 DSC Evaluation of Cetylpyridinium Chloride for the Reduction of Bacterial Populations on Beef Hide Surfaces

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There is a need to reduce the prevalence and density of pathogenic bacteria on hides as cattle enter the slaughtering floor, and therefore in this study cetylpyridinium chloride (CPC; 1%) was evaluated for its ability to reduce *Escherichia coli* O157:H7 and total bacterial populations on beef hide surfaces. Hide samples inoculated with 8 log CFU/ml of a five-strain mixture of *E. coli* O157:H7 were immersed (0.5 min) in the CPC solution and allowed to stand for 0.5 to 30 min prior to neutralization with Dey-Engley broth. Exposure to CPC for 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 or 30.0 min before neutralization reduced *E. coli* O157:H7 populations by 2.1, 3.0, 2.9, 2.8, 3.2, 2.7 and 3.3 log CFU/cm^2, respectively; comparable reductions in total plate counts (initially 8.2 log CFU/cm^2) were 1.6 and 2.7 log CFU/cm^2 when the samples were neutralized 0.5 and 30.0 min, respectively, after exposure to CPC. In an additional study, exposure to CPC ranged from 0.5 to 5.0 min and then the samples were neutralized either 5.0 or 10.0 min following exposure. When neutralized 5.0 min after exposure to CPC, *E. coli* O157:H7 populations were reduced ($P < 0.05$) by 1.9, 2.6, 3.2 and 3.8 log CFU/cm^2 following 0.5, 1.0, 2.5 and 5 min of exposure to CPC, respectively; greater ($P < 0.05$) reductions were observed when the CPC was neutralized 10 min after treatment application. The application of a CPC solution on animal hides would likely contribute to the effectiveness of decontamination interventions used during the slaughtering process and could prove to be beneficial in reducing the prevalence and extent of contamination with *E. coli* O157:H7.

T49 Trends of *Salmonella* Serotypes in the United States: FoodNet, 1996 to 2001

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An estimated 1.4 million cases of *Salmonella* occur each year in the United States. Better understanding the epidemiology of common serotypes will help target prevention efforts. To determine how the incidence of common *Salmonella* serotypes has changed from 1996 to 2001, we reviewed data from the Centers for Disease Control and Prevention's Foodborne Diseases Active Surveillance Network (FoodNet). Data include laboratory-confirmed cases of *Salmonella* occurring within FoodNet sites (13% of the US population). Poisson regression was used for trend analysis. Among *Salmonella* isolates serotyped in 2001, the most frequently isolated *Salmonella enterica* serotypes in

FoodNet sites were Typhimurium (25%), Enteritidis (15%), Newport (12%), Heidelberg (7%), and Javiana (5%). Incidence per 100,000 persons, by serotype in 2001, was: *S.* Typhimurium 3.3; *S.* Enteritidis 2.0; *S.* Newport 1.6; *S.* Heidelberg 0.9; and *S.* Javiana 0.7. From 1996 to 2001, trend analysis noted decreases in *S.* Typhimurium (24%) and *S.* Enteritidis (22%), and increases for *S.* Newport (32%), *S.* Heidelberg (34%), and *S.* Javiana (228%). Declines in *S.* Typhimurium and *S.* Enteritidis, the two most frequently isolated serotypes, and increases in *S.* Newport, *S.* Heidelberg, and *S.* Javiana, suggest risk factors and exposures to different reservoirs of salmonellosis may be evolving. The declines suggest success with meat, poultry, and egg food safety programs, although continued vigilance is required. Further exploration of current risk factors for *S.* Newport, *S.* Heidelberg, and *S.* Javiana infection is needed.

T50 Persistence of *Salmonella* Enteritidis PT4 and S.Typhimurium DT104 on a Commercial Laying Farm

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Salmonella Enteritidis, especially phage type (PT) 4, is the predominant cause of human salmonellosis and is often linked with consumption of raw or undercooked egg products. In recent years there has been a reduction of *S.* Enteritidis infection in humans, up to 2001 when there was a small upturn. The reduction in infection has corresponded with introduction of vaccination of laying flocks, and improved hygiene and egg storage. The presentation describes an investigation which is in progress. The aim of this work is to carry out intensive sampling of 3 poultry flocks over time. Each of the flocks on the farm has been infected with both *S.* Enteritidis PT4 and *S.* Typhimurium DT104 simultaneously. The organisms are also being carried by mice and flies. After cleaning and disinfection of the poultry houses a significant level of persistent *Salmonella* was found and subsequent flocks have become infected. Sampling has demonstrated a high level of contamination of the environment with both organisms, despite vaccination with a killed *S.* Enteritidis bacterin. Both *Salmonella* strains were found in feces, in dust, on egg belts and in spillage from egg belts within the houses but no DT104 was found in the egg packing plant and on the surfaces and in contents of eggs, even though it was widespread within the poultry houses. Similarly, during passage of sterile eggs through the packing plant, only *S.* Enteritidis PT4 was acquired. A non-motile variant of *S.* Enteritidis was found in spent hens from one flock at post-mortem but other flocks were infected with motile organisms.

T51 Detection and Enumeration of *Salmonella* Enteritidis in Ice Cream Associated with an Outbreak: Comparison of Conventional and Rapid Methods

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Over the last two decades, *Salmonella* Enteritidis (SE) has become an increasing public health problem throughout the world. Many of the outbreaks caused by SE are attributable to the consumption of products containing raw or undercooked eggs such as homemade ice cream. Government agencies are seeking to identify farm-to-table actions that will decrease the food safety risks associated with shell eggs based on risk assessment. However, SE risk assessment is limited by the lack of both qualitative and quantitative data, mainly because of the low number of SE present in shell eggs and egg products and inefficient analysis methodology. Traditional cultural methods for the detection and enumeration of SE are slow and laborious. Therefore, specific, sensitive, and rapid methods for SE are needed to collect sufficient data for risk assessment and food safety policy development. We developed a real-time quantitative polymerase chain reaction (PCR) assay for direct detection and enumeration of SE and applied it to naturally contaminated ice cream samples. In this assay, a segment (97-bp) of the gene sef14 specific to *Salmonella* group D strains, such as *Salmonella* Enteritidis and *Salmonella* Dublin, was amplified by polymerase chain reaction (PCR). The amplification of the target gene products was monitored in real-time by incorporating fluorescent dye-labeled gene-specific probes in the PCR reaction. When applied to direct detection and quantification of SE in ice cream, the real-time PCR assay was as sensitive as the conventional plate count method, but real-time quantitative PCR-derived cell numbers were one to three logs higher than both most probable numbers and colony-forming units obtained by conventional culture methods. However, the detection and enumeration of SE in naturally contaminated ice cream can be completed in 4 h using the developed real-time PCR method, while cultural enrichment methods require 5 to 7 days. The real-time PCR assay proved to be a valuable tool for collecting data for SE risk assessment, and may be useful for the food industry in monitoring its processes to improve product quality and safety.

T52 DSC Longitudinal Studies on *Listeria* in Smoked Fish Plants: Impact of Employee Training and Intervention Strategies on Contamination Patterns

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Four ready-to-eat (RTE) smoked seafood plants were monitored for 2 years to study *Listeria* contamination patterns and the impact of employee training and targeted sanitation intervention strategies on the incidence of *Listeria* in the processing plant environment, and in raw and finished product. Samples collected monthly were tested for *Listeria* spp. and *L. monocytogenes*. There was considerable variation in contamination rates and patterns for individual plants. For year 1, a total of 56 raw product (20.3%, n=276), 24 finished product (8.7%, n=275) and 162 environmental (26.3%, n=617) samples tested positive for *Listeria* species. Early in year 2, intensive employee training was performed and targeted *Listeria* intervention strategies were implemented in all plants. A total of 46 raw product (19.0%, n=242), 16 finished product (6.6%, n=244) and 103 environmental (19.5%, n=527) samples were positive for *Listeria* species in year 2. Frequency of environmental *Listeria* contamination showed statistically significant improvement for non-food contact surfaces (from 35% to 17%). There was no significant reduction in *Listeria* contamination levels of floor drains, food contact surfaces or employee contact surfaces, especially in the raw processing areas of the plants. Environmental contamination by *L. monocytogenes* was most prevalent in floor drains (49.6% and 54.2% of drain samples testing positive for years 1 and 2, respectively). Specific *L. monocytogenes* ribotypes predominated in two plants, DUP-1053A and DUP-1039A (50% and 64% of *L. monocytogenes* positive samples in each plant, respectively). Our data indicate that employee training and targeted intervention can reduce *Listeria* environmental contamination, which likely contributes to RTE product contamination. Additional efforts appear to be necessary to eliminate persistent *L. monocytogenes* subtypes.

T53 DSC The Effects of Soil and Surface-type on the Survival of *Listeria monocytogenes* in the Presence of Condensate

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The survival of *Listeria monocytogenes* (LM) on three surfaces used commonly in food processing environments was evaluated at 10°C in the presence of condensate with and without organic soil [as brain-heart infusion broth (BHIB)]. The surfaces included stainless steel, DuPont Delrin® acetal resin (sometimes used for parts of various food processing equipment – e.g. slicers), and fiberglass reinforced plastic (FRP) wall paneling. Cells were attached to both sides of 25.8 cm^2 coupons (total surface area, 51.6 cm^2) by immersion in a five strain LM cocktail (10^7 CFU/mL) in phosphate buffer (unsoiled) or diluted BHIB (soiled). After attachment, coupons were rinsed with sterile distilled water and then either soiled (sprayed with BHIB), or left unsoiled and then placed into sealed petri dishes wherein 100% relative humidity was ensured throughout the study. Coupons were incubated at 10°C. Cells were removed from coupons by agitation in phosphate buffer with glass beads and then enumerated by plating onto Tryptic Soy Agar at multiple times over a 15 day period. The $\log_{10}$ CFU of LM per soiled and unsoiled coupons was 6.5–6.9 and 4.1–5.1 immediately after attachment, respectively. LM populations did not decline over a 15 day period on BHIB soiled coupons but were undetectable at 9 days (stainless steel), and 11 days (acetal and FRP). The data indicate the importance of organic matter removal (e.g. during cleaning) in food manufacturing facilities wherein condensate formation may occur.

T54 Effect of Inoculum Size on the Growth/No Growth Boundary of *Listeria monocytogenes*

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The effect of initial inoculum density on the limits of temperature, substrate pH and a_w allowing growth of *Listeria monocytogenes* was studied. Growth initiation of *L. monocytogenes* in tryptic soy broth was studied in 2000 combinations of temperature (4 to 30°C), pH (3.76–6.44), a_w (0.888–0.997) adjusted with NaCl, and inoculum level (8.0×10^0 to 6.5×10^6 CFU/microtiter plate well). Growth was detected by recording turbidity (620 nm) of broth using an automated microplate reader. Absence of growth was confirmed by plating of the broth on tryptic soy agar and comparing resulting populations with the inoculum size using Student's t-test. The results indicated that effects of pH, a_w and incubation temperature levels in limiting growth of the pathogen were affected by density of the initial inoculum. For example, at 25°C and a_w of 0.997, the minimum pH values where growth was observed were 4.45 and 3.94 for inoculum levels of 8.0×10^0 and 6.5×10^6 CFU, respectively, while at 25°C and pH 5.96 the minimum a_w values of growth for the above inoculum levels were 0.928 and 0.900, respectively. The growth/no growth data were further modeled using a logistic regression process, and a growth/no growth interface model for *L. monocytogenes* as a function of temperature, pH, a_w and inoculum size was developed. The results indicated that *L. monocytogenes* cell populations are not homogeneous and suggest that growth limits of cells are best represented by distributions. The developed model was used as a basis for the evaluation of variability in growth limits and for the description of *L. monocytogenes* populations by distributions.

T55 Withdrawn

T56 Withdrawn

T57 FSIS *Listeria* Risk Assessment: Dynamic In-plant Model to Evaluate the Effectiveness of Testing Food Contact Surfaces

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Foodborne listeriosis represents a relatively rare but serious disease of significant public health concern. While a variety of foods may be contaminated by *Listeria monocytogenes* (Lm), outbreaks and sporadic cases of listeriosis are predominately associated with ready-to-eat (RTE) foods. Two such products, hot dogs and deli meats, were recently identified as those that pose a medium and high risk of listeriosis, respectively. There is evidence that a source of Lm in these products is due to post-processing environmental contamination. To protect public health, FSIS proposed to require that establishments without HACCP controls for Lm test food contact surfaces (FCS) for *Listeria* spp. (Lspp) with a minimum frequency based on the establishment's size. To evaluate the effectiveness of testing and sanitizing FCS for Lspp and the reduction in the risk of listeriosis, FSIS developed a dynamic in-plant Monte Carlo risk assessment model for Lm in hot dogs and deli meats. In order to examine this relationship, the model takes into consideration the frequency and amount of Lspp on FCS and the transfer of Lspp to RTE product. The ratio of Lspp to Lm was based on published data. The level of Lm on RTE product was calibrated based on recently available NFPA data. The outputs of this dynamic model were linked to the FDA/FSIS risk ranking model which takes the product from the plant to the table in order to estimate the risk of illness. Results from this risk assessment were used to guide FSIS risk management decision-making for RTE products.

T58 Withdrawn

T59 Application of a Safety Monitoring and Assurance System for Minimizing the Risk of Listeriosis Associated with Cooked Ham

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A safety monitoring and assurance system (SMAS) for minimizing risk of listeriosis associated with cooked ham at the time of consumption is presented. In this system, instead of the conventional first-in first-out (FIFO) method, a new approach based on actual risk evaluation at important points of the chill chain is used in order to promote products to the next stage of distribution. This evaluation is based on continuous product temperature monitoring and the use of predictive models for the growth of *L. monocytogenes*. The applicability of SMAS is demonstrated and evaluated based on *L. monocytogenes* kinetics and chill chain data employing the Monte Carlo simulation method. Furthermore, the effectiveness of SMAS on the spoilage status of cooked ham at the time of consumption was evaluated based on the growth of *Lactobacillus sakei*, chosen as the specific spoilage organism. In order to simulate the results of the application of the SMAS system a chilled chain scenario is used, consisting of production, transportation to the main distribution center, transportation to the local market (2 to 24 h) or export market (24 to 72 h), 6, 24 or 36 h in retail storage and various time periods in the consumer's refrigerator. Two decision points are used to apply the SMAS approach. At the first decision point, the main distribution center, products are appropriately split and sent to the close local market or the distant export market based on *L. monocytogenes* growth. At the second decision point, units are classified into 3 groups for successive stocking of the retail cabinets every 6 h with the products with higher growth of *L. monocytogenes* promoted first. Without the use of SMAS, product split at the above two points with the common FIFO approach is random, since time in the chill chain for all products in consideration is the same. For the local market the risk distribution of products distributed based on SMAS and FIFO approach was found to be similar. For the export market however, SMAS application led to a substantial shift of the central tendency of the risk distribution to lower risk probabilities (from 10-7 with FIFO to 10-8 with SMAS) and a significant decrease of products with high risk. Furthermore, for the export market with the FIFO system 12.54% of products were spoiled at the time of consumption, whereas with SMAS unacceptable products were reduced to 4.32%.

T60 Predictive Modeling of Spoilage of Fresh Meat: The Effect of Temperature and Modified Atmosphere Packaging

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Spoilage of chilled fresh and minimally processed meat products is attributed mainly to bacterial activity. Modelling the growth of the fraction of microflora responsible for spoilage as a function of storage conditions, mainly temperature, is essential in shelf life prediction and control. The growth of the spoilage bacteria *Pseudomonas* spp., lactic acid bacteria and *Brochothrix thermosphacta*

was monitored and modeled as a function of temperature (0 to 15°C) and the concentration of carbon dioxide in aerobic as well as in CO_2 enriched packaging atmospheres (up to 100%) of fresh meat. Shelf life of samples was assessed for all experimental cases by the Quality Index Method. Combined models were developed and comparatively assessed based on polynomial, Belehradek, and Arrhenius equations. The activation energy parameter of the Arrhenius model, EA, was independent of the packaging atmosphere and ranged from 60 to 80 kJ/mol, whereas biologically meaningful estimates for parameters, such as CO_2 max (for bacterial growth) and Tmin (for bacterial growth) were also evident. Developed models were evaluated by comparing predictions of growth rates of Specific Spoilage Organisms (SSOs) from independent literature and laboratory data on meat with those predicted by the developed models under the same conditions of storage as those of the present study. Models indicated good overall performance for all SSOs and enabled reliable prediction of shelf-life to a next stage.

T61 Quantifying Robustness of a Microbial Growth Model

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A methodology for assessing robustness of a microbial growth model is proposed. This method involves computing a robustness index (RI) as the ratio of the standard error of prediction (SEP) to the standard error of calibration (SEC) for a given model, where RI near 1 indicates a robust model. The SEC was defined as the root mean square error of the growth model against the data ($\log_{10}$ CFU/g vs. time) used to generate the model. SEP was defined as the root mean square error of the model against an independent data set. The use of this technique was illustrated by evaluating the robustness of the USDA-ARS Pathogen Modeling Program, which was developed from broth-based data, in predicting the growth of *E. coli* O157:H7 in ground beef at different conditions. For example, comparisons against previously published data from experiments in ground beef at 20°C, 5.8 pH, and 0.99 water activity yielded estimated RI values from 1.7 to 1.8, while data from 37°C, 5.8 pH, and 0.99 water activity yielded estimated RI values from 3.9 to 4.1. Assessing the robustness of a microbial growth model is essential in order to ascertain its predictive ability for future cases, and the RI is a quantitative measure for evaluating the relative robustness of various models under different conditions.

T62 Bayesian Synthesis of a Pathogen Growth Model

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Bayesian synthesis is proposed as one possible means of evaluating the robustness of predictive microbiology models. The Bayesian synthesis method is motivated by means of an empirical example, applying the Baranyi growth model to data from two studies on the growth of *Listeria monocytogenes*. The Bayesian synthesis method was first proposed to characterize uncertainty in mechanistic process models (e.g., of population dynamics). The basic approach is to generate a joint premodel distribution on all model inputs and outputs. A joint postmodel distribution on the inputs and outputs is then generated by importance sampling. The joint postmodel distribution can be marginalized to obtain a postmodel distribution of any quantity of interest. Interval estimates are available from the marginalized posterior distributions, as well as the estimated correlation structure among the model parameters. A key feature of Bayesian synthesis is that it assumes that premodel information on the inputs and outputs is available from independent information, as are the observed data used to update the model. If the location of the posterior distribution is substantially shifted from that of the stated prior, this raises questions as to the robustness of the prior results relative to the independently observed data. In the example presented here, however, the posterior is not substantially shifted, and synthesis of the independent sources of information about *L. monocytogenes* growth results in reduced uncertainty in the Baranyi model predictions.

T63 Fuzzy and Statistical Techniques for Food Safety Risk Assessment

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In applying risk assessment methods to food systems, there is a need to deal with uncertainties in terms of system behaviour and control effects. Survival of *Campylobacter jejuni* through a sequence of poultry processing operations was simulated to compare statistical (probability-based) and fuzzy methods for defining uncertainty and variability in parameter values. Interval arithmetic with fuzzy values considers all possible combinations in calculations and maximum membership grade for each possible result. Consequently fuzzy results fully included distributions estimated by Monte Carlo simulations but extended to broader limits (i.e., estimates are more conservative). This is probably a benefit in early stage risk assessment

when there is often limited data to define probability distributions for all inputs. Fuzzy simulation is computationally simple relative to the sampling design and iterative calculations that are part of Monte Carlo methods. Whereas Monte Carlo results vary somewhat with each simulation run, fuzzy calculations produce identical numerical results for every simulation. As long as the model does not become too complex (i.e., a long chain of calculations), the mean of a fuzzy simulation is reasonably close to a Monte Carlo model based on the same information. Fuzzy simulation techniques can be used at the outset and probability-based measures added as more reliable information becomes available.

T64 Risk Assessment in Pork Production: Modeling Porkborne *Salmonella* Risk from Farm to Pork

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Bacterial foodborne diseases cost $5.6 to $9.4 billion in the U.S. annually. Meat and poultry account for 80% of these costs (Buzby, 1996). We have summarized the conceptual framework for a risk assessment for porkborne *Salmonella* to humans (Barber, Miller, and McNamara, forthcoming *JFP*, 2003). The paper outlined here reports the quantitative risk model and results to date. With the model, hazards are identified and characterized, exposure is assessed, and risk characterization problems regarding porkborne *Salmonella* are addressed. Our study uses @Risk, an add-on program to Excel, to trace the chain of *Salmonella* risk from farm to table. Input variables are in five modules: swine production, transportation, lairage, slaughter and pork retail, and consumption. Input variables were defined using existing scientific literature. Output variables are embedded throughout these modules and also in the sixth module, the human health risk assessment module. These modules are connected together to conduct a series of analyses in the overall risk assessment. Three sets of analyses are conducted. First, the number of human *Salmonella* cases attributable to pork is estimated. Second, key factors that appeared to have the most influence on the resulting human cases were identified. Third, various mitigation scenarios are simulated. Preliminary analyses show that the number of human *Salmonella* cases attributable to contaminated pork is not high. Nevertheless, the long right tail of the distribution illustrates possible high values especially with breaks in mitigation strategies. Consumption and preparation, especially cooking, is important in decreasing *Salmonella* risk with farm level controls of lesser importance.

T65 Quantitative Risk Assessment of *Vibrio parahaemolyticus* in Bloody Clams in Southern Thailand

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A microbiological risk assessment was conducted in order to estimate risk of *Vibrio parahaemolyticus* (Vp) infection in southern Thailand. There have been very few epidemiological data in relation to Vp infection or contamination in this region. However, a preliminary study suggested that the characteristics of the clinical strains in this area were consistent with those isolated from shellfish rather than other seafood. Therefore, this risk assessment was focused on a popular bivalve in Thailand, the bloody clam (*Anadara granosa*). Exposure assessment was divided into four stages; harvest, retail, cooking and consumption. Samples were obtained from a harvest site and the same batch of the clams was kept at ambient temperatures to simulate local evening markets and dinner cooking situations. At harvest and retail stages, the total numbers of Vp were assumed to have lognormal distribution, and were modeled by the Bootstrap method. The prevalence of pathogenic strains (tdh + and trh+) in these stages was estimated by Bayesian inference. The prevalence of total Vp after boiling was estimated from the laboratory generated data. A beta-Poisson dose-response model developed in the FAO/WHO risk assessment for Vp in oysters was used in the hazard characterization. Finally, the probability of illness following consumption of a single serving of a clam dish was estimated for a defined population. Although there are many limitations and assumptions, this project serves as a sample study for the first-step data generation and modeling operated by Thailand-Japan international team.

T66 Quantitative Risk Assessment for Transmission of *Cryptosporidium* or *Giardia* in Norway by Consumption of Contaminated Mung Bean Seed Sprouts

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Environmental and animal feces surveys have indicated that *Cryptosporidium* and *Giardia* infections are widespread in Norway. However,

human infection with these parasites continues to be considered non-endemic. No central reporting system for cryptosporidiosis exists in Norway, unless combined with a diagnosis of Acquired Immune Deficiency Syndrome. A recent survey of vegetable and fruit samples in Norway demonstrated that mung bean sprouts were significantly more likely than the 9 other produce types analyzed to be contaminated with *Cryptosporidium* oocysts or *Giardia* cysts. Using prevalence and concentration information from a Norwegian study, a dose-response was applied in a quantitative risk assessment. The resulting probability of infection from *Cryptosporidium* or *Giardia*, from ingestion of contaminated mung bean sprouts in Norway, was calculated to be 12.7 and 16.4 per 100,000 population respectively. The highest rate of giardiosis reported in Norway was 8.3 cases per 100,000 population in 1999, mostly attributed to travel abroad. The assessment conservatively assumes that all infection results in illness. Reported incidence rates for giardiosis are probably substantially underestimated due to under-reporting and asymptomatic infection. No incidence data is available for cryptosporidiosis in the general population. Data gaps identified in the analysis include not only information on the extent of infection, but also bean sprout consumption data, infectivity and genotype of parasites, and effects of immunity and repeated exposure. Nevertheless, this analysis suggests that acquisition of these infections via consumption of contaminated mung bean sprouts might be of concern within Norway, and elsewhere.

T67 Biogenic Amines Production by Bacteria Isolated from Herring (*Clupea harengus*)

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One hundred twenty bacterial strains were isolated from fresh herring, and herring spoiled after storage without ice, in modified atmosphere packs (MAP) and in vacuum packs (VP) and investigated for their abilities to produce biogenic amines in amino acid decarboxylase broths. A rapid HPLC method was used for detection of biogenic amines using a gradient elution program with acetonitrile and water. The microflora of fresh herring was dominantly *Pseudomonas* (30%), *Enterobacteriaceae* (23.2%), *Vibrio* (13.3%) and *Moraxella* (13.3%). However, the microflora of herring stored in VP and MAP was dominated by species belonging to *Vibrio* (23.3%) and *Moraxella* (20%). In amino acid decarboxylase broths, not all bacterial strains produce the biogenic amines but most of them produced histamine, putrescine and cadaverine. The highest histidine decarboxylase activities were observed in *Klebsiella oxytoca*, *Hafnia alvei* and *Proteus vulgaris*, which produced 396 mg and 232 mg and 54 mg histamine/L, respectively, in histidine-enriched broth. The accumulation of cadaverine by *K. oxytoca* and *H. alvei* was 325 mg/L and 208 mg/L, respectively. All strains isolated produced putrescine in an ornithine-enriched broth, ranging from 3 mg/L to 249 mg/L. The production of putrescine by *K. oxytoca* and *H. alvei* was 249 mg/L and 195 mg/L, respectively.

T68 Mechanistic Dose-response Modeling for Microbial Risk Assessment

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Microbial dose-response models are based on human feeding trial data if available. Such trials may carry an unacceptably high risk and may not be representative of susceptible populations. Therefore, for most microorganisms, accurate data on infectivity do not exist. A workshop was held in December 2002 to identify ways to improve microbial dose-response modeling. Participants discussed the use of mechanistic models that consider the biological factors that affect the likelihood that an ingested organism will cause infection and disease, and identified the following events as relevant when developing such models: 1. History of the pathogen prior to consumption; 2. Gastric effects on microbial survival and infectivity; 3. Intestinal attachment and infection; 4. Survival of host defenses including immune challenge; and 5. Adverse responses. Many factors need to be considered when developing mechanistic dose-response models, including presence of normal gut microflora, previous exposure, immune status, and nutrition status. At the cellular level, the immunological response is very complex. A major challenge is to develop mechanistic models that are meaningful but not excessively complex, as they may be unworkable. Well-characterized animal models will be useful to improve understanding of microbial dose-response, but their utility for human health risk assessment depends on the ability to extrapolate to humans. In vitro systems may offer the potential to isolate and characterize components of the model. Biologically based mechanistic dose-response models appear possible with advances in understanding the processes of infection, disease and immune response. Such models can potentially provide better estimates of human health risks.

Symposium Abstracts

S01 Use of Food Safety Objectives and Other Risk-based Approaches to Reduce Foodborne Listeriosis

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Listeria monocytogenes is a foodborne pathogen that can cause listeriosis, a severe disease, which symptoms include septicemia, meningitis, and spontaneous abortion. The rate of listeriosis has declined over the past 10 years, and while the current level of listeriosis is fairly low (around 0.3 cases per 100,000 population) this disease has a high fatality rate: of the estimated 2,500 cases in the United States each year, approximately 500 people die from the illness. The ILSI Risk Science Institute convened an Expert Panel to consider how to ensure further reductions in foodborne listeriosis, using a risk-based approach. The Expert Panel met three times over the past year, both in plenary session and in break-out groups, to review the state of the science. The Panel considered how to set food safety objectives for this organism, and how control strategies might be used to reduce listeriosis rates associated with food. The findings of the Expert Panel will be presented in this symposium.

S02 Intervention Strategies for Ready-to-Eat Meat Products

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The meat and poultry industry has available to it a series of intervention technologies that can assist in the production of safe ready-to-eat (RTE) foods. These strategies can involve the use of post-packaging thermal pasteurization, product formulation interventions, and non-thermal processing technologies. At present, product formulations to inhibit pathogens are favored by many processors while post-packaging thermal pasteurization and non-thermal treatments, such as high pressure processing (HPP), are used by a few of the larger companies. Thermal and non-thermal interventions offer a viable means of producing safe RTE foods by destroying pathogens while additive interventions act by inhibiting the organism's growth. All three systems have benefits and drawbacks. Thermal pasteurization can offer a cost effective, high throughput system for some product types, such as whole muscle products and poultry deli products, while non-thermal treatments such as HPP can effectively treat sliced luncheon meats. In order for the intervention strategies to have maximum effect they must be coupled with an effective plant sanitation program that includes aspects of plant layout, equipment design and appropriate use of sanitizing agents. Finally, to ensure the level of pathogen control necessary to achieve the desired public health goals, processors should consider the use of food safety objectives. Food safety objectives specify goals that can be incorporated into the design of control measures for the production and preparation of foods. This symposium will provide information on several intervention strategies available to processors. The concept of food safety objectives will be discussed with respect to establishing and validating the effectiveness of the available control measures.

S03 Hazard Identification in the Fresh Produce Industry

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Consumer health and well-being are strongly influenced by the food we eat, and fresh fruits and vegetables play a very important role in promoting good health. In recent years, however, fresh produce has been associated with a number of widely publicized outbreaks of foodborne illness. Great attention has been placed on minimizing microbial food safety hazards in fresh produce, perhaps most significant of which was the publication of a "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables". This document sponsored by the US Department of Health and Human Service and the Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN), is now available in four languages. Although the industry has largely embraced this guidance, practical and economically effective programs are limited by gaps in our understanding of risk potential. Additional research is needed to identify the risks that exist in the diverse production, packing, fresh-cut processing and distribution operations. It is only with an accurate understanding of microbiological hazards to the produce industry that we can effectively reduce and/or control contamination. This symposium will focus on soil amendment safety, survival of human pathogens on fresh produce, farm worker risk assessment, the prevalence of various indicator organisms and pathogens in fresh produce, and hazards identified in FDA farm investigations. We will also review the likelihood of occurrence of pathogens in fresh-cut produce, and risk assessment in transportation and distribution of produce.

S04 Recipe for Food Safety at Retail

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Today, families and individuals are obtaining a greater number of meals from retail food establishments. And when consumers eat food from a retail establishment, they expect and have a right for it to be safe. Clearly, for many products, the responsibility for food safety at the retail level is shared among several different groups in the food production chain. Nevertheless, the retail industry must and will continue to do its part. Producing and serving safe food at retail can involve a variety of complex issues. They range from the continual need to educate and train an ever-changing workforce, to a variety of regulatory compliance issues that can differ depending on the state or region, to dealing with special dietary requests from patrons with food allergies. Accordingly, this symposium will review several challenges involved in producing safe food at retail, proven strategies used to effectively manage food safety risk and recent innovations in retail food safety.

S05 Effective Food Worker Hygiene Interventions: A Risk Assessment Approach

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Commercial food workers play a significant role in transmission of infection caused by foodborne pathogens. This symposium reviews

results from a number of studies related to foodworker hygiene, including EHS-Net focus group data obtained from food workers and managers concerning facilitators and barriers to safe food handling. Research on the relative risk associated various transmission routes and contributing causes of outbreaks attributed to foodworker malpractices are the basis for prioritizing interventions. In order to determine which intervention measures and approaches are the most effective for improving food handler behavior, different personal hygiene strategies were explored, taking into consideration variability and uncertainty using quantitative microbial risk assessment tools. Results of this risk assessment help enable optimization of both messages and how they should be communicated. Methods used to overcome compliance issues (barriers) are particularly relevant to key intervention approaches, and a training model was developed with emphasis on the behavioral and psychological aspects incorporating elements of social marketing. Benefit-cost analyses, performed in infection control activities in healthcare and food industries, show these approaches are cost-effective. Food worker problems in developing countries are different from those in developed countries and future risk assessment and cost benefit approaches with proper training should be applied to them.

S06 Investigative Molecular Techniques and Their Application to Food Safety

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Applications of molecular biology continue to be incorporated into the field of food safety. This symposium focuses on the various roles that molecular biology methodologies play in food safety, now and in the future. This includes various techniques that are increasingly sensitive and specific for detecting and identifying foodborne viruses, parasites and bacteria. Bacterial subtyping methods can not only facilitate the detection of foodborne disease outbreaks, but also represent tools to track sources of bacterial contamination throughout the farm to fork continuum. Classical phenotypic subtyping methods have been important tools for detection of foodborne disease outbreaks for many years. The last 5 to 10 years have seen tremendous advancements in the development of more sensitive, more rapid, and increasingly easy-to-use (and sometime even automated) molecular subtyping methods. The development of bioinformatics, computer-based, and electronic exchange technology allows for a variety of different bacterial foodborne pathogens to be analyzed, with rapid exchange of bacterial subtype data. Assumptions must often be made in the nascent science of microbial risk assessment. Information obtained from techniques based on molecular biology will aid epidemiologists, government and regulatory agencies, and policy makers. In some cases such data may challenge the validity of often made assumptions. In the last few years, DNA microarray technology has emerged as one of the most powerful tools for global profiling of gene expression. Microarray technology provides a significant advancement in our ability to generate genomic fingerprints and expression profiles of foodborne pathogens under various conditions. These techniques provide the food industry with tools that help provide a more focused approach to food safety.

S07 Current Issues in the Microbiological Safety of Dairy Foods — From Farm to Table

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Considering their widespread use and distribution, dairy products in recent years are rarely associated with significant outbreaks due to foodborne microbial pathogens. Safety issues have been raised however, regarding the occurrence of potential risk organisms on the farm and in the plant, the relevance of current microbial standards for dairy products and the impact of changes in processing procedures. For the most part regulatory, industry and academia have been pro-active in addressing these concerns. Regarding dairy farms, organisms with the potential for human heath concerns (i.e. *Salmonella*, *E. coli*, the causative

agent of Johnes – *M. paratuberculosis*) occur, but can be controlled with appropriate biosecurity and health assurance measures. Once removed from the farm, raw milk and manufactured dairy products must meet microbiological standards established by FDA, USDA or other governing agencies. These standards, some of which were established decades ago, are currently under review in regard to their relevance to current food safety questions. The safety considerations for cheeses manufactured from raw or sub-pasteurized milk are also under review, although these products have historically been considered safe with proper handling and aging. Other cheese safety questions have been generated from the increased interest in farmstead, artesian and certain culture specific cheese products. In general, improved processing and sanitation procedures are required for increased dairy product safety. Fluid milks and other products are being processed for longer shelf life (i.e. utilizing high heat, improved packaging), which warrants evaluation of these products under extremes of distribution and handling conditions. As procedures and standards for the dairy industry change from farm to table, the microbiological safety of dairy products will continue to present challenges in regard to implementation and the need for continued research and review.

S08 Hot Topics in Seafood Quality and Safety

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Seafood comprises numerous species of fish and shellfish, which provide nutritional benefits to the consumer. Although per capita consumption has leveled in the United States, consumers are advised to increase their diet of seafood. However, concerns have been directed at knowing if the seafood products are safe. In this symposium, a range of topics focusing primarily on current microbiological questions is covered. Overviews of chemical contamination and genetically-modified foods complete this forum.

S09 New Horizons in Diagnostic Food Microbiology

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Since the times of Louis Pasteur, microbiologists have continually strived to improve microbiological methods. In this seminar, we will review many of the changes in methods which have increased sensitivity, specificity, speed, and range of detectable organisms. We will also examine how these changes enable microbiological methods to provide more and better information which is essential to produce foods which are safe and meet ever changing regulatory criteria. Specific technologies which will be discussed include real-time PCR, biosensors, biochip and microarray technologies, and the use of molecular methods as alternatives to traditional serotyping. Finally, because methods are changing so rapidly at the same time that the global economy is bringing food companies and consumers worldwide closer together, the area of standardization and harmonization of methods will be discussed.

S10 Food Allergens: Past, Present, and Future

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Food allergies affect more than six million people in the United States. It has been estimated

that 150 people die each year as a result of food-induced anaphylaxis. Strict avoidance of allergen-containing foods remains the only successful method to manage food allergies. Consumers with food allergies rely on food labels to disclose the presence of allergenic ingredients. However, undeclared allergens can be inadvertently introduced into food by cross-contamination either during manufacturing in processing plants or during preparation in restaurants or other food service establishments. As reported by the FDA, the number of food recalls for undeclared allergens rose steadily during the 1990s and continues to be one of the leading causes of food recalls. Many of these recalls were the result of equipment cross-contact contamination by undeclared allergens. In recent years, food processors and the food service industry have implemented improved allergen controls measures to prevent such occurrences. This symposium will provide an overview of the past, present and future issues and concerns regarding food allergens. An update on current and emerging analytical methods for food allergen detection will be given. An historical look at the regulation of food allergens, a review of current FDA guidance, and an outlook on future regulations of food allergens will be presented. There also will be discussions on the principles of equipment design for effective and sanitary removal of food allergens. Food processors will be sharing their experience regarding the cleaning, validation and verification procedures for the removal of allergens from equipment. The best practices developed by the food service industry to prevent inadvertent allergen contamination will also be presented.

S11 Costs of Industry and Government Food Safety Actions: What is at Stake?

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Foodborne bacterial pathogens add tremendous costs to the economy. There are the costs in human suffering, lost productivity and sometimes death to individuals who get sick from contaminated foods. There are the added costs to the producer and processor of implementing testing programs and interventions designed to eliminate or significantly reduce the presence of any pathogens on their products. There are the potential costs of recalls of products with the subsequent potential loss of consumer confidence in the company's products. There is the cost of litigation from consumers who became sick or died from eating contaminated foods. This symposium is designed to present the economic aspects of each of these elements in order to help the producer, processor and consumer develop a better understanding of the true costs of food safety.

S12 Spoilage and Pathogenic Fungi and Yeasts

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Fungi have been used for thousands of years for the production of fermented foods and beverages and have contributed immensely to human health and prosperity. Fungi also have detrimental impacts on human health through infections and by the production of mycotoxins, which when consumed by animals and humans may have serious health consequences. Fungi also have a tremendous economic impact in the spoilage of foods. In this symposium, biological properties of fungi and their economic impact on the food industry are described. Contemporary methods for detection of fungi are discussed. A study of the interactions of fungi with pathogenic bacteria is described. An emphasis is given in the symposium to mycotoxins and their control during food production and processing. Novel molecular methods to understand biosynthesis of mycotoxins and to control mycotoxigenic fungi and mycotoxins in crops are discussed. The outstanding potential for the use of genomics in analyzing fungal physiology and the discovery of novel antifungal agents is described. This symposium will provide an overview of spoilage and mycotoxigenic fungi and yeasts as well as descriptions of novel approaches for detection and control of fungi in foods.

S13 Assuring Food Safety and Security

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The potential for contamination of our food supply has always been present. The food industry has responded with sound food production, processing, and distribution practices to minimize this threat. Now a rise in terrorism has increased the potential for food contamination. Sound security practices and timely information is needed to minimize this threat to public safety and the food industry. The United States has a multitude of farms, food processors, and retail food facilities that supply food to the public. Unfortunately, biological, chemical, radiological, or physical agents can be used to contaminate food at the farm, during transport, or during and after food processing. A bioterrorism threat or act directed toward our food supply could create a public health crisis. The goal of this symposium is to inform the audience of the current biological terrorism threat and risks to the food industry. The symposium will also address methods to decrease the risk of an event by addressing food security, risk management, and threat identification.

S14 Applied Microbiological Genomics for Food Safety and Quality

STANLEY BRUL, University of Amsterdam/ Unilever Research, Swammerdam Institute for Life Sciences, Nieuwe Achtergracht 166, 1018 WV, Amsterdam, The Netherlands; S. NOTERMANS, TNO Nutrition and Food Research Institute, P.O. Box 360, 3700 AJ Zeist, The Netherlands; PAUL TAKHISTOV, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA

The food processing industry is continuously faced with an ever-increasing demand for safe, tasty and wholesome food. To meet these demands many new products, using new and preferably mild processing technologies and preservation systems, are being developed. The classical approach to test products for safety is time consuming and limited by the fact that the response of the microorganisms cannot be measured directly. During the past several years a new genomics-driven tool has been developed which makes it possible to measure directly all basic responses of microorganisms in one assay. This system, known as micro-array technology, offers completely new possibilities. Examples, among others, are genomics-driven preservation and genomics-based predictive microbiology. The last approach may even allow predicting the storage life of a food product without knowing its composition. Other fields of application include: the identification of novel microbial compounds, prediction of the optimal state of maturity of fruit, and control of fermentation processes. During the session a general introduction will explain genomics technology and related micro-array systems and their possible application in the food safety and quality area. This will be followed by descriptions of two practical applications of the micro-array system: one dealing with predictive microbiology based on a genomics approach and the second with cell-based assays and biosensors as new tools for detection and quantification in food microbiology.

S15 *Campylobacter*: A Pathogen in Need of Resolution

ROBERT V. TAUXE, CDC, 1600 Clifton Road, Mail Stop A-38, Atlanta, GA 30333, USA; ERIC LINE, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30604-5677, USA; KELLI HIETT, USDA-ARS-RRC, P.O. Box 5677, Athens, GA 30604-5677, USA; MICHAEL ROBACH, Wayne Farms LLC, 4110 Continental Drive, Oakwood, GA 30566, USA; RUFF LOWMAN, Canadian Food Inspection Agency, Animal Disease Research Institute Rm C317, 3851 Fallowfield Road, Nepean, ON K2H 8P9, Canada

Campylobacter jejuni/coli is the most frequently reported bacterial causative agent of foodborne human gastroenteritis. Because *Campylobacter* rarely causes death, its importance has been relegated relative to other more lethal agents. The most frequently associated vehicle for human infection has been poultry, which is often contaminated by the organism. The published literature suggests that *Campylobacter* infection occurs most frequently as sporadic cases. In some settings, typing strategies can identify dispersed clusters of infection that relate common sources which would otherwise not be observed among the many sporadic cases. In spite of the strong association with poultry, targeted interventions have not yet eliminated health concerns. Qualitative and quantitative methods have been published which describe approaches to evaluate flocks of poultry and resulting commercial products. Total elimination from poultry seems distant, but adequate reduction in public exposure may dramatically reduce risk for infection. The purpose of this symposium is to bring together knowledgeable individuals possessing experience and insight to several pertinent disci-

plines that are needed to create an approach to control transmission of *Camylobacter.* The following topics shall be presented as components of the symposium: sources for the organism as determined by human case-control studies; current cultural methods required for both qualitative and quantitative assessments of products; developing and available non-cultural methods for *Campylobacter* detection, characterization and applications of these methods; goals of the poultry industry relative to *Campylobacter* and; and risk assessment as a tool to understand *Campylobacter* presence and levels in the environment and in poultry operations which contribute to its transmission.

S16 Microbial Stress Response to Intervention Technologies

AUBREY F. MENDONCA, Iowa State University, Dept. of Food Science and Human Nutrition, 2313 Food Sciences Bldg., Ames, IA 50011, USA; JOHN S. NOVAK, USDA-ARS-ERRC, Microbial Food Safety Research Unit, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; HOWARD Q. ZHANG, Ohio State University, Dept. of Food Science and Technology, 2015 Fyffe Court, Room 110, Columbus, OH 43210, USA; GARY P. RICHARDS, Delaware State University, James W. W. Baker Center, Room 29, 1200 N. Dupont Hwy., Dover, DE 19901, USA; DALLAS G. HOOVER, University of Delaware, Dept. of Animal and Food Science, Room 017 Townsend Hall, Newark, DE 19717, USA

Increasing numbers of consumers desire foods with higher qualities. Consequently, improvements upon food processing methods in use today are often envisioned to reduce the need for chemical additives, increase safety from pathogens or spoilage microorganisms, and give a perception of minimal treatment or processing. Concurrently foodborne illnesses in the United States are estimated to exceed 76 million cases annually resulting in economic losses of 5 to 6 billion dollars. Newer technologies often attempt to offer advances over existing practices used by the food industry. The topics in this symposium question the effectiveness of processing technologies still in development regarding treatment of foodborne microorganisms and responses of microorganisms should survival following the process stress occur. Technologies in development to be addressed include gamma irradiation, ozone, pulsed-electric field, and high pressure processing. Susceptibility of food pathogens to the technologies will be assessed from the most recent food laboratory studies.

S17 Current Issues in Food Toxicology

ROBERT HOLLINGWORTH, Michigan State University, Center for Integrated Plant Systems, 206 CIPS, Wilson Road, East Lansing, MI 48824, USA; MICHAEL BOLGER, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA; JAMES C. GRIFFITHS, Burdock Group, Toxicology and Risk Assessment, 780 US Hwy. One, Suite 300, Vero Beach, FL 32962-1660, USA; PENELOPE A. FENNER-CRISP, ILSI Risk Science Institute, One Thomas Circle, NW, 9th Floor, Washington, D.C. 20005, USA

Emerging developments in the area of food toxicology and associated food safety standards and regulations will be reviewed and discussed by world-recognized food toxicology experts. Topics will include: (1) a review of the safety determination of foods-derived through biotechnology, (2) a review of the standards and regulations that govern the use of bioactive substances and functional food ingredients, (3) safety standards for foodborne contaminants, and (4) the use of toxicological data from human subjects in regulatory policy. Speakers will provide an overview of the topics, including time for questions from the audience. Attendees can expect to gain a broad appreciation of some of the emerging key topics in food toxicology, and the implications for food safety standards, policies, and regulations.

S18 Science-based Shelf-life Dating of Ready-to-Eat Refrigerated Foods

JILL HOLLINGSWORTH, Food Marketing Institute, 655 15th St. NW, Suite 700, Washington, D.C. 20005, USA; MICHAEL P. DOYLE, University of Georgia, CFSQE, 1109 Experiment St., Griffin, GA 30223-1797, USA; RICHARD C. WHITING, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; MARK W. CARTER, Kraft Foods NA, 801 Waukegan Road, Glenview, IL 60025, USA; TED LABUZA, University of Minnesota, Dept. of Food Science and Nutrition, 136 ABLMS, St. Paul, MN 55108, USA; ROY P. BETTS, Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD, UK

Industry has historically established date codes primarily based on quality attributes of food products. However, recently there has been interest in considering a scientific basis for establishing safety-based code dates for refrigerated ready-to-eat foods that support the growth of psychrotrophic pathogens such as *Listeria monocytogenes, Clostridium botulinum,* and others. This issue has been fueled in part by recent outbreaks of listeriosis and risk assessments that demonstrate risk increases

with higher levels of *L. monocytogenes*. This symposium will explore issues such as the microbiological concerns, the criteria that can be applied to determine if date labeling can be a useful food safety tool, protocols that can be used to establish a scientifically valid, safety-based date code, and whether alternatives such as time/temperature integrators could be useful. In addition the symposium will look at the history of shelf-life dating and consumer perceptions about date coding and provide a European perspective on the issue.

S19 All the Latest Jazz — Recent Foodborne Disease Outbreaks

SAM GOTTLIEB, CDC, 1600 Clifton Road NE, NCID-DBMD-Mailstop A38, Bldg. 3 – B49, Atlanta, GA 30333, USA; DAVID GOLDMAN, USDA, 1400 Independence Ave. SW, Room 334, Washington, D.C. 20250, USA; KATRINA KRETSINGER, CDC, 1600 Clifton Road NE, NCID-BMD-Mailstop D63, Dec 1-5011, Atlanta, GA 30333, USA; J. DOUGLAS PARK, FDA, Room 2C029, HFS-600, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; LARRY BEUCHAT, University of Georgia, CFSQE, 1109 Experiment St., Griffin, GA 30223-1797, USA; KEVIN WINTHROP, California Dept. of Health Services, Tuberculosis Control Branch, 2151 Berkeley Way, Berkeley, CA 97404, USA; MARY PALUMBO, California Dept. Health Services, 601 N. 7th St., MS 357, P.O. Box 942732, Sacramento, CA 94234-7320, USA; SHERRI MCGARRY, FDA-OFP, HFS-605, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA

Many people hear rumors about foodborne disease outbreaks that have occurred, but they never see a final report or journal article that describes the investigations, their findings and conclusions. This symposium will include three epidemiological reports of recent foodborne disease outbreaks presented by CDC representatives who were involved in the investigations. The symposium will also include three environmental investigation reports from the same or similar outbreak investigations. The presentations will demonstrate the complexity of the investigations along with findings that can be used to inform future prevention efforts. This brief abstract is being written in mid-January. At this writing we have an enthusiastic commitment from Dr. Rob Tauxe to supply the three epidemiological investigation speakers, but the final decision has not been made on which outbreaks to present. We are considering the series of cantaloupe outbreaks that have occurred in recent years along with recent tomato and lettuce outbreaks. We will work with Dr. Tauxe in the next few weeks to make final decisions on the epidemiological investigations and then line up the appropriate environmental investigation papers to go with them. This symposium is a place marker for what we see as a kind of late-breaking symposium where we try and present as current information as we can. This approach makes it hard to have specifics this far in advance of the actual presentations.

S20 Food on the Move

DEAN DAVIDSON, FDA-CFSAN, Interstate Travel Program, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; DAVID L. FORNEY, CDC, National Center for Environmental Health/Vessel Sanitation Program, 4770 Buford Hwy. NE/MS F16, Atlanta, GA 30341-7333, USA; CPT., CHARLES S. OTTO, CDC, National Center for Environmental Health/Vessel Sanitation Program, 4770 Buford Hwy. NE/MS F28, Atlanta, GA 30341-3724, USA; JULIE BUTNER, Compass Group, North American Div., 2309 Colonial Pkwy., Fort Worth, TX 76109, USA; MARC-ALAIN WIDDOWSON, CDC, Viral Gastroenteritis Section, Respiratory and Enteric Viruses Branch, Prevention Mail Stop G04, 1600 Clinton Road, Atlanta, GA, 30333, USA; TONY POMETTO, NASA Food Technology, Commercial Space Center, Iowa State University, 2901 South Loop Drive, Suite 3700, Ames, IA 50010-8632, USA

The traveling public consumes millions of meals and snacks each month. Food is served on planes, on ships, on trains, on buses and even in space. Preparation, holding, storage and service aboard moving conveyances present unique challenges. Outbreaks of bacterial and viral illnesses have, in the past, been documented among airline passengers and, more recently, aboard cruise ships. This past year, a number of cruise ships have faced major illness outbreaks, causing significant monetary loss and damage to their reputations. A number of federal organizations are responsible for food safety in this milieu. The FDA's Interstate Travel Program regulates both the food and the environmental aspects aboard interstate conveyances. The CDC's Vessel Sanitation Program is a "fee-for-service" voluntary program specifically for the cruise industry, using a modified FDA Food Code as its basis. Both organizations have oversight roles, but the companies making and serving the food have the ultimate responsibility for food safety. This symposium will describe the activities of the federal agencies, examine some of the recent outbreaks, and showcase some of the steps the industries are taking to apply HACCP to assure food safety.

S21 Aquaculture: Safety and Quality Issues

JUAN SILVA, Mississippi State University, Food Science and Technology Dept., Mississippi State, MS 39762, USA; CASEY GRIMM, USDA-ARS-SRRC, 1100 Robert E. Lee Blvd., New Orleans, LA 70179-0687, USA; ROSALIE SCHNICK, Michigan State University, National Center Regional Aquaculture Center, 3039 Edgewater Lane, LaCrosse, WI 54630, USA; CHARLES SANTERRE, Purdue University, Dept. of Foods and Nutrition, Stone Hall, Room 205, 700 W. State St., West Lafayette, IN 47907-2059, USA; PETER K. BEN EMBAREK, WHO, Food Safety Programme, 20 Ave. Appia, Geneva, CH-1211 27, Switzerland

Both in the Untied States and internationally, aquaculture is a fast-growing sector of the freshwater and marine food industry. Consumers are advised to eat more fish as a health benefit. An opposing view is expressed for limiting consumption of these foods due to potential health concerns related to chemical contaminants. This symposium bridges the need for more, high quality aquacultured fish and seafood while acknowledging potential safety risks.

S22 The Evolution of Foodborne Pathogens

TOM A. CEBULA, 8301 Muirirk Road, Laurel, MD 20708, USA; TOM S. WHITTAM, Michigan State University, NFSTC, 165 Food Safety and Toxicology Bldg., East Lansing, MI 48824, USA; ANDREAS J. BAEUMLER, Texas A&M University, Dept. of Medical Microbiology and Immunology, 407 Reynolds Medical Bldg., College Station, TX 77843-1114, USA; MARTIN C. J. MAIDEN, University of Oxford, Dept. of Zoology, Peter Medawar Bldg., Oxford 0X2 3SY, UK; MARTIN WIEDMANN, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853, USA

The long-term survival of bacteria reflects its capability to selectively adapt to ecological niches favorable to its persistence and replication. Recent studies of selected bacteria pathogenic for humans have shown the evolution of particular traits which contribute to virulence and/or host adaptation. This symposium will provide an overview of the evolution of foodborne pathogens, and of the techniques and approaches used to study their molecular evolution. Recent work on *Escherichia coli* O157: H7, *Salmonella* Enteriditis and *Listeria monocytogenes* will be discussed in the context of what it shows us about the molecular evolution of these organisms as foodborne pathogens. The insights from this expanding field of investigation may provide new ways and/or new targets for designing strategies for control of foodborne pathogens.

S23 Natural Antimicrobials — Current Trends and Future Perspectives

P. MICHAEL DAVIDSON, University of Tennessee, Dept. of Food Science and Technology, 2509 River Drive, Knoxville, TN 37996, USA; ERIC A. JOHNSON, University of Wisconsin-Madison, Food Research Institute, 1925 Willow Drive, Madison, WI 53706, USA; SADHANA RAVISHANKAR, NCFST, IIT Moffet Campus, 6502 S. Archer Road, Summit-Argo, IL 60501, USA; JENNIFER GROWER, Clemson University, B212 Polle Ag Center, Box 340370, Clemson, SC 29630, USA; JOSEPH D. MEYER, Kraft Foods, 200 DeForest Ave., East Hanover, NJ 29636, USA; BARBARA J. PETERSEN, Novigen Sciences, Inc., 1730 Rhode Island Ave. NW, Suite 1100, Washington, D.C. 20036, USA

Food preservation to render processed food products safe for human consumption remains a challenge for the food industry and regulatory agencies. One of the strategies used to achieve food preservation is the use of naturally occurring bio-derived compounds, thereby inhibiting growth of undesirable microorganisms. The use of these compounds is gaining popularity in recent years due to a variety of reasons. There is a possibility of resistance development in bacteria towards a particular chemical compound. Consumers are concerned about the safety of synthetic additives in foods, due to the increased awareness about health and diet, and about the risks arising from use of chemical preservatives. These bio-derived compounds considered natural by consumers can be classified into three categories; those derived from microorganisms, plants and animals. These compounds may exhibit antimicrobial activity in the foods in which these are normally found or may be used as hurdles in multifactorial food preservation systems. Researchers have investigated the antimicrobial activity of many of these compounds. Each has a specific inhibitory spectrum against a variety of microorganisms. Current trends also include the incorporation of antimicrobials into materials used for packaging food products and this is one area that needs further investigation for commercial applications. While no single antimicrobial can serve to preserve a product that is grossly contaminated alone, the use of combinations of natural antimcrobials and processes is likely to remain an important strategy in food preservation to meet consumers demand for wholesome and safe foods.

S24 Risk Communication — Putting Food Safety in Perspective

DAVID ROPEIK, Harvard Center for Risk Analysis, 718 Huntington Ave., Boston, MA 02115, USA; CAROLYN O'NEIL, Carolyn O'Neil Productions, 995 W. Kingston Dr., Atlanta, GA 30342, USA; CARY FRYE, International Dairy Foods Association, 1250 H St. NW, Suite 900, Washington, D.C. 20005, USA; DANIEL H. JOHNSON, JR., Clearview Medical Imaging, 3100 Clearview Pkwy., Metairie, LA 70006, USA; DAVE SCHMIDT, International Food Information Council, 1100 Connecticut Ave. NW, Suite 430, Washington, D.C. 20036, USA

Food safety continues to be a topic of great interest and debate among science experts, the media, government and public health officials, and most importantly consumers. Communication about the risks associated with food as well as the positive benefits of food and certain food components has been a growing challenge among industry, government and science experts alike. Recent media attention, advocacy and misinformation concerning Bovine Somatotropin (BST) and dairy products, acrylamide in foods, methylmercury and fish consumption as well as various recalls of meat products, has become an overwhelming challenge for consumers to understand. For example, anti-dairy activists have attempted to spread misinformation about the health and safety of dairy products. The role of third party experts has helped to put these issues in perspective. Effective risk communication should include sensitivity toward public perception about the issue at hand. A clear understanding of "hot button" issues can help shape and focus an effective communication strategy. Appropriate risk communication will include announcements, contextual information, and instructions from the expert community to various audiences including the media and consumers. It should raise the level of understanding on relevant issues and adequately satisfy the public with the most current, accurate and available information. For any communication strategy to be successful, trust must be established. Proactive communication from government, industry and science experts to consumers about risks can lead to trust among the public. Through this risk communication symposium, we will attempt to enhance communication skills for attendees who interact with the media and consumers.

S25 Emerging Issues in Water Quality for the Food Industry

SUSAN MCKNIGHT, Quality Flow, Inc., 3691 Commercial Ave., Northbrook, IL 60062, USA; KELLY A. REYNOLDS, University of Arizona, Environmental Research Laboratory, 2601 E. Airport Drive, Tucson, AZ 85706, USA; JEANETTE THURSTON-ENRIQUEZ, USDA-ARS, 138 Keim Hall, East Campus, University of Nebraska-Lincoln, Lincoln, NE 68583-0934, USA; JIM VAN VOOREN, Environmental Health Laboratories, Underwriters Laboratories, 110 South Hill St., South Bend, IN 46530, USA; ADRIAN PETERS, University of Wales Institute-Cardiff, Llandaff Campus, Cardiff, Wales CF5 2YB, UK; KRISTINA D. MENA, University of Texas Health Science Center at Houston, School of Public Health, 1100 N. Stanton, Suite 110, El Paso, TX 79902, USA; PETER M. KENNEDY, Quality Flow Inc., 3691 Commercial Ave., Northbrook, IL 60062, USA

Water is a key ingredient that impacts food as it travels from farm-to-table, yet it is either missing from most food safety discussions or just mentioned as an aside. The Water Quality & Safety Professional Development Group was formed in 2002 to explore the impact water quality has on the food chain. This is the first symposium of the Water Quality & Safety PDG and we will begin by identifying some problems surrounding water quality and safety for food purveyors, as well as some solutions. There will be a discussion of how surface and ground waters both have water quality problems, even after the treatment plant. These problems have been shown to result in illness. We will be exploring issues that impact irrigation water quality, how biofilms impact the safety of food products and how much of an impact water quality has in foodborne disease. Methods for analyzing water quality and treating water problems in the food industry will also be reviewed. Water impacts all parts of the food chain and this symposium begins to identify issues that affect the true potability, quality, and safety of water from start to finish, on a global basis.

Abstract Book Addendum

as of **August 4, 2003**

MONDAY MORNING – AUGUST 11, 2003

(S02) Facility Design and Sanitation Best Practices – Oliver Reelsen, Wayne Chemical Co., Fort Wayne, IN, USA will be a co-presenter.

(T05) Evaluation of Second Generation VIDAS® *Listeria monocytogenes* and Automated BAX® Methods for Detection of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry – will be presented by Ann Marie McNamara, Silliker, Inc., Homewood, IL, USA

(T06) Validation of a Microwell DNA Probe Assay for Detection of *Listeria* spp. in Foods – will be presented by Mark A. Mozola, Neogen Corporation, Lansing, MI, USA

MONDAY AFTERNOON – AUGUST 11, 2003

(T22) Improving Urgent Public Health Information Dissemination in California: The Food Safety Notification System – Speaker last name change – Jennifer Thomas changed to Jennifer Bruns

(P062) Withdrawn

(P063) Withdrawn

(P087) Withdrawn

TUESDAY MORNING – AUGUST 12, 2003

(S10) Food Allergens and the Food Service Industry – will be presented by Steven F. Grover, National Restaurant Association, Washington, D.C., USA

(T33) A Field Study of the Microbiological Quality of Fresh Produce – Speaker last name change – Lynette Kleman changed to Lynette Johnston

(T36) The Use of Gradient Plates to Study the Combined Effect of Temperature, pH and NaCl Concentration on the Growth of *Monascus rubber* van Tieghem, an Ascomycetes Fungus Isolated from Green Table Olives – will be presented by Panagiotis N. Skandamis, University of Athens, Athens, Votanikos, Greece

TUESDAY AFTERNOON – AUGUST 12, 2003

(S13) Assuring Food Safety and Security – Organizer/Convenor last name change – Jennifer Thomas changed to Jennifer Bruns

(S13) Scientific and Technological Approaches for Counter-terrorism of Foods – will be presented by Bob Buchanan, FDA-CFSAN, College Park, MD, USA

(S13) Bio-Security North Carolina – John T. Hoffman, North Carolina Dept. of Agriculture and Consumer Services, Raleigh, NC, USA will replace the 2:30 presentation.

(S13) The USDA Perspective on Bioterrorism Prevention and Response – will be presented by Don Musacchio, USDA-FSIS, Washington, D.C., USA

(S14) Applied Microbiological Genomics for Food Safety and Quality – Ian Jenson will be convenor

WEDNESDAY MORNING – AUGUST 13, 2003

(S19) Multistate Listeriosis Outbreak Associated with Turkey Deli Meat – United States, 2002 – will be presented by Sami Gottlieb, CDC, Atlanta, GA, USA and Kristin G. Holt, USDA-FSIS-OPHS-HHSD, Atlanta, GA, USA

(S20) The New Worldwide Food Safety Standards for the Airline Industry – will be presented by Carol Heaver Norton, Flying Food Group, Jamaica, NY, USA

(T55) Potential for Underestimation of *Escherichia coli* O157:H7 Prevalence in Beef Feedlot Cattle – G. H. LONGERAGAN, M. M. Brashears, and G. Dewell, West Texas A & M University, Canyon, TX, USA

(P183) Characterization of a Swarming Phenotype of *Listeria innocua* on Semi-solid Surfaces – Correct spelling for presenter is Emmanouil Apostolidis

(P198) Withdrawn

(P215) Withdrawn

WEDNESDAY AFTERNOON – AUGUST 13, 2003

(S22) Evolution of *Salmonella* Virulence and Host Adaptation – Correct spelling for presenter is Andreas J. Baumler

(S22) MLST (Multilocus Sequence Typing) for Evolutionary Analyses and Outbreak Tracking – will be presented by Kate E. Dingle, University of Oxford, Oxford, UK

(P282) Withdrawn

(P284) Monitoring of Total Volatile Basic Nitrogen, Trimethylamine Nitrogen and Biogenic Amines in Salted and Dried Chub Mackerel – will be presented by Joaquin Rodrigo, University of Ciudad, Juarez, Chihuahua, Mexico

(P286) Baseline Risk Study of Chemical Contaminants in Ontario Farm-raised Rainbow Trout – will be presented by Mike Cassidy, Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada