

Journal of Food Protection

ISSN: 0362-028X
Official Publication



International Association for
Food Protection
Formerly IAMFES
Reg. U.S. Pat. Off.

Vol. 63

July 2000

No. 7

Molecular Beacon Polymerase Chain Reaction Detection of <i>Escherichia coli</i> O157:H7 in Milk	John L. McKillip and MaryAnne Drake*	855
Evaluation of the Reveal and SafePath Rapid <i>Escherichia coli</i> O157 Detection Tests for Use on Bovine Feces and Carcasses	Christine A. Power,* Roger P. Johnson, Scott A. McEwen, W. Bruce McNab, Mansel W. Griffiths, W. Ronald Osborne, and Stephanie A. De Grandis	860
Commercial Field Trial Evaluation of Mucosal Starter Culture To Reduce <i>Salmonella</i> Incidence in Processed Broiler Carcasses	J. S. Bailey,* N. J. Stern, and N. A. Cox	867
Reduction of <i>Salmonella</i> spp. and Strains of <i>Escherichia coli</i> O157:H7 by Gamma Radiation of Inoculated Sprouts	Kathleen T. Rajkowski* and Donald W. Thayer	871
Attachment and Growth of <i>Salmonella</i> Chester on Apple Fruits and In Vivo Response of Attached Bacteria to Sanitizer Treatments	Ching-Hsing Liao* and Gerald M. Sapers	876
Combined Effects of Hydrostatic Pressure, Temperature, and the Addition of Allyl Isothiocyanate on Inactivation of <i>Escherichia coli</i>	Tetsuro Ogawa,* Atsushi Nakatani, Hajime Matsuzaki, Seiichiro Isobe, and Kenji Isshiki	884
Antimicrobial Activity of a 14-Residue Synthetic Peptide against Foodborne Microorganisms	Paola Appendini and Joseph H. Hotchkiss*	889
Effectiveness of Two Cooking Systems in Destroying <i>Escherichia coli</i> O157:H7 and <i>Listeria monocytogenes</i> in Ground Beef Patties	Elaine M. D'Sa, Mark A. Harrison,* Scott E. Williams, and Marc H. Broccoli	894
Characterization of <i>Vibrio parahaemolyticus</i> Isolates Obtained from Foodborne Illness Outbreaks during 1992 through 1995 in Taiwan	Hin-Chung Wong,* Shu-Hui Liu, Lee-Wen Ku, I-Ying Lee, Tien-Kuei Wang, Yeong-Sheng Lee, Chih-Lung Lee, Li-Ping Kuo, and Daniel Yang-Chih Shih	900
Growth and Survival of <i>Escherichia coli</i> O157:H7 and <i>Listeria monocytogenes</i> in Egg Products Held at Different Temperatures	Shu-Er Yang and Cheng-Chun Chou*	907
Vancomycin Resistance and Antibiotic Susceptibility of Enterococci in Raw Meat	Maria Pavia, Carmelo G. A. Nobile, Letterio Salpietro, and Italo F. Angelillo*	912
Behavior of <i>Listeria monocytogenes</i> in Pasteurized Milk during Fermentation with Lactic Acid Bacteria	Wayne M. Pitt,* Terence J. Harden, and Ron R. Hull	916
Use of Capillary Tubes and Plate Heat Exchanger to Validate U.S. Department of Agriculture Pasteurization Protocols for Elimination of <i>Listeria monocytogenes</i> in Liquid Egg Products	C. B. Michalski, R. E. Brackett,* Y.-C. Hung, and G. O. I. Ezeike	921
Growth of <i>Bacillus cereus</i> on Solid Media as Affected by Agar, Sodium Chloride, and Potassium Sorbate	Mara Lucia Stecchini,* Manuela Del Torre, Stefania Donda, and Enrico Maltini	926
Isolation and Identification of Nontuberculous Mycobacteria from Foods as Possible Exposure Sources	Claudia Argueta,* Sean Yoder, Alan E. Holtzman, Timothy W. Aronson, Norman Glover, O. George W. Berlin, Gerard N. Stelma, Jr., Seymour Froman, and Paul Tomasek	930
Effects of Gamma Radiation on Sensory Qualities, Microbiological and Chemical Properties of Salted and Fermented Squid	Myung-Woo Byun,* Kyong-Haeng Lee, Dong-Ho Kim, Jae-Hun Kim, Hong-Sun Yook, and Hyun-Joo Ahn	934
Effects of Gamma Radiation on the Conformational and Antigenic Properties of a Heat-Stable Major Allergen in Brown Shrimp	Myung-Woo Byun,* Jae-Hun Kim, Ju-Woon Lee, Jung-Won Park, Chein-Soo Hong, and Il-Jun Kang	940
Genotoxicity Testing of Cooked Cured Meat Pigment (CCMP) and Meat Emulsion Coagulates Prepared with CCMP	M. Stevanović,* P. Čadež, B. Žlender, and M. Filipič	945
Qualitative Detection of Tetracycline Residues in Milk with a Luminescence-Based Microbial Method: The Effect of Milk Composition and Assay Performance in Relation to an Immunoassay and a Microbial Inhibition Assay	Jussi Kurittu,* Stefan Lönnberg, Marko Virta, and Matti Karp	953

Research Notes

Isolation of <i>Salmonella</i> spp. from the Housefly, <i>Musca domestica</i> L., and the Dump Fly, <i>Hydrotaea aeneascens</i> (Wiedemann) (Diptera: Muscidae), at Caged-Layer Houses	Alan R. Olsen* and Thomas S. Hammack	958
Efficacy of Alkaline Washing for the Decontamination of Orange Fruit Surfaces Inoculated with <i>Escherichia coli</i>	Steven Pao,* Craig L. Davis, and D. Frank Kelsey	961
A Survey of Water Activity and pH Values in Fresh Pasta Packed under Modified Atmosphere Manufactured in Argentina and Uruguay	Carolina Schebor and Jorge Chirife*	965
Buffalo-Milk Enzyme Levels, Their Sensitivity to Heat Inactivation, and Their Possible Use as Markers for Pasteurization	P. Lombardi, L. Avallone, A. d'Angelo, T. Mor, and E. Bogin*	970
Survey of the Furosine Content in Cheeses Marketed in Spain	Mar Villamiel,* María Arias, Nieves Corzo, and Agustín Olano	974

Reviews

Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects	C. J. Greenwalt,* K. H. Steinkraus, and R. A. Ledford	976
Criteria to Determine Food Allergen Priority	Jupiter M. Yeung,* Rhona S. Applebaum, and Regina Hildwine	982

* Asterisk indicates author for correspondence.

The publishers do not warrant, either expressly or by implication, the factual accuracy of the articles or descriptions herein, nor do they so warrant any views or opinions offered by the authors of said articles and descriptions.

Research Note

Isolation of *Salmonella* spp. from the Housefly, *Musca domestica* L., and the Dump Fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at Caged-Layer Houses

ALAN R. OLSEN* AND THOMAS S. HAMMACK

U.S. Food and Drug Administration, HFS-315, 200 C Street S.W., Washington, D.C. 20204, USA

MS 99-362: Received 2 December 1999/Accepted 1 February 2000

ABSTRACT

Flies, especially houseflies, are widely recognized as potential reservoirs and vectors of foodborne *Salmonella* pathogens. In this study, flies were collected at caged-layer facilities that had produced eggs that were implicated as the food vehicle in two recent outbreaks of *Salmonella* Enteritidis infections. The flies were separated by species into pools for microbiological testing. A total of 15 species pools of houseflies, *Musca domestica* L., and 7 species pools of bronze dump flies, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), were analyzed. *Salmonella* Enteritidis was isolated from 2 of the 15 pools of houseflies. Other species of *Salmonella* were isolated from three pools of flies, including *Salmonella* Infantis from houseflies and from dump flies and *Salmonella* Heidelberg from houseflies. *Salmonella* Mbandaka was isolated from a lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae).

Within the past 10 years, eggs have emerged as a major source of foodborne infections from *Salmonella* Enteritidis (14, 15). Flies, especially houseflies (*Musca domestica* L.) (Diptera: Muscidae), are proven carriers of foodborne pathogens that affect humans (9). In the poultry industry, the greatest numbers of houseflies and other disease-carrying flies occur in caged-layer houses (poultry houses with laying hens in cages for commercial egg production), where the flies breed in accumulated manure beneath the cages (6). The flies that breed in layer houses are a potential reservoir of pathogens, including *Salmonella* Enteritidis (3, 6, 8). There is no prior research, however, investigating possible links between flies and actual outbreaks of foodborne *Salmonella* Enteritidis infections. This report contains the results of microbiological analyses of flies that were captured at caged-layer house facilities whose poultry eggs were identified as the food vehicle in two recent outbreaks of *Salmonella* Enteritidis infections.

The two separate outbreaks occurred in 1998, affecting 46 and 26 people who became ill from *Salmonella* Enteritidis after eating foods that contained egg. In both cases, epidemiological investigators identified eggs as the implicated ingredient in the food vehicles for the *Salmonella* Enteritidis infections. The egg ingredients were traced to specific caged-layer facilities. The U.S. Food and Drug Administration (FDA) sent teams to the facilities to collect environmental samples. During these investigations, flies were collected at each facility to determine if the flies were

a potential contributing factor to the spread of *Salmonella* Enteritidis.

MATERIALS AND METHODS

Collection of flies. Live adult flies were collected at two different caged-layer house facilities (facility 1 and facility 2) that were identified as a potential source of contaminated eggs. The houses were suspected of housing *Salmonella*-infected flocks of hens whose eggs were implicated in the outbreaks of salmonellosis. With one exception, the flocks were caged above pits in which manure from the layer hens was allowed to accumulate. The exception was house A, facility 1, which was equipped with a conveyor system for the immediate removal of manure from the house. All the houses contained laying hens in cages that were stacked in tiers up to eight cages high inside the houses. Traps were placed at various locations inside and outside each caged-layer house. Each trap was exposed for approximately 15 min and then aseptically placed in a separate sterile plastic container. In addition, separate collections of flies were made at each trap site using a hand net. The netted specimens were pinned and identified to serve as voucher specimens for this research. The voucher specimens were not used in *Salmonella* analyses.

Fly specimens from each glue board trap were aseptically pooled according to species and trap. Specimens were sorted by species using morphological characters observed at $\times 10$ to $\times 20$ magnification (7, 8). Table 1 summarizes the species, number of flies, and location of capture for each species pool from the glue board traps.

Isolation of *Salmonella* from flies. The pools of fly specimens were separately analyzed for the presence of *Salmonella* according to the *Bacteriological Analytical Manual* culture method for raw flesh foods, highly contaminated foods, and animal feeds (5). Housefly samples (less than 1 g) were pre-enriched in

* Author for correspondence. Tel: 202-205-4438; Fax: 202-205-4091; E-mail: aolsen@bangate.fda.gov.

TABLE 1. *Species pools of flies collected at caged-layer houses*

Pool no.	Fly species	No. of specimens	Collection location
1	<i>M. domestica</i>	4	Facility 1, house A, egg conveyer
2	<i>M. domestica</i>	8	Facility 1, house A, outside rear door
3	<i>H. aenescens</i>	1	Facility 1, house A, outside rear door
4	<i>M. domestica</i>	3	Facility 1, house A, inside rear door
5	<i>M. domestica</i>	4	Facility 1, house B, egg conveyer
6	<i>M. domestica</i>	6	Facility 1, house B, inside manure pit
7	<i>H. aenescens</i>	1	Facility 1, house B, outside manure pit
8	<i>H. aenescens</i>	1	Facility 2, house A, inside house
9	<i>M. domestica</i>	1	Facility 2, house A, outside manure pit
10	<i>H. aenescens</i>	1	Facility 2, house A, outside manure pit
11	<i>M. domestica</i>	5	Facility 2, house A, outside manure pit
12	<i>H. aenescens</i>	5	Facility 2, house A, outside manure pit
13	<i>M. domestica</i>	19	Facility 2, house A, in manure pit
14	<i>H. aenescens</i>	7	Facility 2, house A, in manure pit
15	<i>M. domestica</i>	1	Facility 2, house B, outside rear door
16	<i>M. domestica</i>	5	Facility 2, house B, outside rear door
17	<i>H. aenescens</i>	1	Facility 2, house B, outside manure pit
18	<i>M. domestica</i>	2	Facility 2, house B, in manure pit
19	<i>M. domestica</i>	1	Facility 2, house C, outside rear door
20	<i>M. domestica</i>	6	Facility 2, house C, outside rear door
21	<i>M. domestica</i>	1	Facility 2, house C, inside house
22	<i>M. domestica</i>	2	Facility 2, house C, in manure pit

10 ml of lactose broth for 24 ± 2 h at $35 \pm 2^\circ\text{C}$. After pre-enrichment, 1-ml aliquots were subcultured to 10-ml portions of tetrathionate broth, and 0.1-ml aliquots were subcultured to 10-ml portions of Rappaport-Vassiliadis medium. Tetrathionate broth and Rappaport-Vassiliadis medium were incubated for 24 ± 2 h at $43 \pm 0.2^\circ\text{C}$ and at $42 \pm 0.2^\circ\text{C}$, respectively. Incubated tetrathionate broth and Rappaport-Vassiliadis medium were streaked to bismuth sulfite, Hektoen enteric, and xylose lysine desoxycholate agar plates. The plates were incubated for 24 ± 2 h at $35 \pm 2^\circ\text{C}$. Bismuth sulfite agar plates, without typical *Salmonella* colonies, were incubated for an additional 24 ± 2 h at $35 \pm 2^\circ\text{C}$. Presumptive positive colonies were picked to triple sugar iron and lysine iron agar slants. The triple sugar iron and lysine iron slants were incubated for 24 ± 2 h at $35 \pm 2^\circ\text{C}$. Presumptive positive *Salmonella* isolates were screened biochemically with API 20E test kits (bioMérieux, Hazelwood, Mo.). Presumptive positive isolates were confirmed serologically with polyvalent A-I and Vi somatic (O) antisera, specific somatic group (O) antisera, and polyvalent a-z flagellar (H) antisera (Difco Laboratories, Sparks, Md.). *Salmonella* isolates were sent to the FDA's Central Laboratory for Microbiological Investigations for definitive serotyping.

RESULTS AND DISCUSSION

Eighteen traps yielded 22 species pools of flies for analysis. Fifteen pools consisted of houseflies, *M. domestica*,

and seven pools consisted of bronze dump flies, *Hydrotaea (Ophyra) aenescens* (Wiedemann) (Diptera: Muscidae) (Table 1). The 15 pools of *M. domestica* ranged from 1 to 19 adult flies per pool. The seven pools of *H. aenescens* ranged from 1 to 7 adult flies per pool. The flies captured by netting but not used for *Salmonella* analysis uniformly consisted of a mixture of *M. domestica* and *H. aenescens*.

Eighteen of the 22 pools of flies that were analyzed yielded no *Salmonella* isolates. Four pools tested positive for *Salmonella*. *Salmonella* Enteritidis was found in pools 2 and 16, from facilities 1 and 2, respectively. *Salmonella* Infantis was also found in pool 2 (facility 1) and in pool 14 (facility 2). *Salmonella* Heidelberg was isolated from pool 20 (facility 2). Table 2 summarizes the isolations of *Salmonella* spp. from the pools of flies. In addition, *Salmonella* Mbandaka was isolated from a lesser mealworm adult, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), from the manure pit beneath house B, facility 2.

Voucher specimens of *M. domestica* (four male and four female) and *H. aenescens* (four male and four female) that were collected by hand net at the sites are deposited at the U.S. National Museum of Natural History in Washington, D.C. Additional voucher specimens are retained in the FDA repository collection in Washington, D.C.

Flies are widely recognized as potential reservoirs and vectors of foodborne *Salmonella* pathogens (2-4, 12). Compared with other flies, *M. domestica* is a superior host for *Salmonella*, especially *Salmonella* Enteritidis (11, 13). *Salmonella* is transmitted by houseflies through mechanical transmission, in vomitus, and in feces (11). It is known that houseflies are able to transmit *Salmonella* Enteritidis and *Salmonella* Typhimurium to human food (10, 13).

TABLE 2. *Salmonella* spp. isolated from pools of flies

Pool no.	Fly species	No. of flies in pool	<i>Salmonella</i> serotype(s) isolated
2	<i>M. domestica</i>	8	Enteritidis and Infantis
14	<i>H. aenescens</i>	7	Infantis
16	<i>M. domestica</i>	5	Enteritidis
20	<i>M. domestica</i>	6	Heidelberg

The role of houseflies in transmitting *Salmonella* to uninfected layer hens or to fresh eggs is not clear. However, the observed diurnal dispersion patterns of houseflies in caged-layer houses is certainly conducive to the transmission of pathogens by flies to laying hens and to any exposed feed, water, or eggs. For example, Anderson and Poorbaugh (1) report that houseflies are active in and around cages until mid-morning, at which time they move to the manure pit for feeding and ovipositing. In the afternoon, houseflies tend to move outdoors but return in the evening to the layer houses, where they rest in the rafters until the next morning (1). This diurnal dispersion pattern fits the accepted FDA behavioral profile for disease-carrying flies (12).

Our results represent the first published report of an FDA investigation that may link houseflies to outbreaks of *Salmonella* Enteritidis involving eggs as the suspected food vehicle. This report combined with what is known about the capabilities of houseflies to transmit *Salmonella* and about the observed activity patterns of houseflies in caged-layer houses lead us to conclude that houseflies merit consideration as one of the many potential risk factors in the prevention of foodborne *Salmonella* Enteritidis outbreaks involving eggs.

ACKNOWLEDGMENTS

The authors thank Dean E. Wagner for scotyping the isolates and Wallace H. Andrews, PhD, for technical comments. We also thank Marilyn F. Balmer, VMD, Diane R. McDaniel, and Tyra S. Wisecup for their assistance.

REFERENCES

- Anderson, J. R., and J. H. Poorbaugh. 1964. Observations on the ethology and ecology of various Diptera associated with northern California poultry ranches. *J. Med. Entomol.* 1:131-147.
- Angelotti, R. 1973. The report of the FDA *Salmonella* task force. U.S. Food and Drug Administration, Washington, D.C.
- Anonymous. 1969. An evaluation of the *Salmonella* problem. National Academy of Sciences, Washington, D.C.
- Anonymous. 1980. Urban pest management: a report prepared by the Committee on Urban Pest Management. National Academy Press, Washington, D.C.
- Anonymous. 1998. *Bacteriological Analytical Manual*, 8th ed., revision A. Association of Official Analytical Chemists, Gaithersburg, Md.
- Axtell, R. C., and J. J. Arends. 1990. Ecology and management of arthropod pests of poultry. *Annu. Rev. Entomol.* 35:101-126.
- Gagne, R. J. 1991. Flies (Diptera), p. 269-295. In J. R. Gorham (ed.), *Insect and mite pests in food*, vol. 1. U.S. Department of Agriculture, Washington, D.C. Agriculture Handbook no. 655.
- Greenberg, B. 1971. Flies and disease, vol. 1: ecology, classification and biotic associations. Princeton University Press, Princeton, N.J.
- Greenberg, B. 1973. Flies and disease, vol. 2: biology and disease transmission. Princeton University Press, Princeton, N.J.
- Greenberg, B., and A. A. Bornstein. 1964. Fly dispersion from a rural Mexican slaughterhouse. *Am. J. Trop. Med. Hyg.* 13:881-886.
- Greenberg, B., J. A. Kowalski, and M. J. Klowden. 1970. Factors affecting the transmission of *Salmonella* by flies: natural resistance to colonization and bacterial interference. *Infect. Immun.* 2:800-809.
- Olsen, A. R. 1998. Regulatory action criteria for filth and other extraneous materials, III: review of flies and foodborne enteric disease. *Reg. Toxicol. Pharmacol.* 28:199-211.
- Ostrolenk, M., and H. Welch. 1942. The common house fly (*Musca domestica*) as a source of pollution in food establishments. *Food Res.* 7:192-200.
- St. Louis, M. E., D. L. Morse, M. E. Potter, T. M. Demelfi, J. J. Guzewish, R. V. Tauxe, and P. A. Blake. 1988. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. *JAMA* 259:2103-2107.
- Trepka, M. J., J. R. Archer, S. E. Altekruze, M. E. Proctor, and J. P. Davis. 1999. An increase in sporadic and outbreak-associated *Salmonella* Enteritidis infections in Wisconsin: the role of eggs. *J. Infect. Dis.* 180:1214-1219.