

## Short Report: Coliform and *Escherichia coli* Contamination of Desserts Served in Public Restaurants from Guadalajara, Mexico, and Houston, Texas

Karen J. Vigil, Zhi-Dong Jiang, Jaclyn J. Chen, Kathryn L. Palumbo, Thushara Galbadage, Eric L. Brown, Jing Yiang, Hoonmo Koo, Margaret W. DuPont, Charles Ericsson, Javier A. Adachi, and Herbert L. DuPont\*

The University of Texas Health Science Center at Houston Medical School, Houston, Texas; The University of Texas–Houston School of Public Health, Houston, Texas; MD Anderson Cancer Center, Houston Texas; Baylor College of Medicine, Houston, Texas; St. Luke's Episcopal Hospital, Houston, Texas

**Abstract.** Bacterial enteropathogens acquired from contaminated food are the principal causes of travelers' diarrhea (TD). We evaluated desserts obtained from popular restaurants in the tourist city of Guadalajara, Mexico, and Houston, Texas, to determine coliform and *Escherichia coli* contamination levels and presence of diarrheagenic *E. coli* known to be important in TD. Contamination for all organisms was seen for desserts served in Guadalajara restaurants. Desserts should be considered as potentially risky foods for development of TD among international visitors to developing regions of the world.

Diarrhea is the most common illness occurring in international travelers to developing tropical and semitropical regions.<sup>1</sup> Bacterial enteropathogens acquired from contaminated food have been shown to be the causative agent in up to 60% of the cases, with enterotoxigenic *Escherichia coli* (ETEC) and enteroaggregative *E. coli* (EAEC) being the principal causes of illness in most high-risk regions.

Although of unproven benefit, counseling future tourists in the likely safe foods and often unsafe items during high-risk travel is recommended by travel medicine experts. These recommendations center around the consumption of cooked items served steaming hot or fruits than can be peeled.<sup>2</sup> Based on the expected microbial safety of food items very high in sugar such as syrups, jelly, honey, and jam, we sought to determine the safety of popular desserts in one tourist-oriented city of Mexico by determining the presence of coliform bacteria and *E. coli* in desserts as indicators of fecal contamination with disease-producing potential.<sup>3</sup> In addition we cultured the desserts for the presence of ETEC and EAEC. The rate of occurrence of contamination for the Mexican desserts was compared with a similar number of desserts obtained from restaurants in Houston, Texas.

Dessert samples were collected from different restaurants from Guadalajara, Mexico, and from Houston, Texas, between June and September 2007. The temperature of the dessert at the time it was served was obtained using a Pyrex professional digital thermometer, and the type of dessert was recorded including presence of frosting, cream filling, ice cream, or fruits. Approximately 15 g of the dessert core was placed in a sterile plastic bag and put directly into a wide-mouth thermos containing wet ice. Samples collected during the day were processed within 4 hours. If a sample was collected at the evening meal, it was refrigerated at 4°C until processed first thing the next morning in the research laboratory in either city in which the study took place (Guadalajara or Houston).

The samples were diluted in sterile distilled water at a ratio of 1 to 10 and were placed in sterile Whirl-Pak bags (American Scientific Products, Houston, TX) for homogenization in a Stomacher 400 blender (Dynatech Laboratories, Alexandria,

VA). Enteric pathogens were sought including *Shigella*, *Salmonella*, *Campylobacter*, *Aeromonas*, *Plesiomonas*, and *Vibrios* (cholera and non-cholera) using standard methods.<sup>4</sup> Level of coliform growth was determined by plating serial 10-fold dilutions of food suspensions onto 4-methylumbelliferyl-b-D-glucuronide (MUG) Lauryl agar to identify fluorescent colonies. *E. coli* was detected by culturing the samples onto MUG *E. coli* agar with biochemical confirmation using Triple Sugar Iron agar and API 20E (bioMérieux Inc., Hazelwood, MO). Five *E. coli* colonies from each food sample were saved on peptone stabs and were transported to the central research laboratory at the University of Texas–Houston School of Public Health in Houston for detection of heat labile (LT) and heat stable (ST) toxins of ETEC by polymerase chain reaction (PCR)<sup>5</sup> and EAEC by HEP-2 cell adherence assay.<sup>6</sup>

Wilcoxon rank sum test was used to test continuous variables.  $\chi^2$  or Fisher exact tests were used to compare categorical variables as appropriate. The analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

A total of 49 dessert samples were collected from 35 restaurants in Guadalajara, and the same number of desserts from 33 restaurants in Houston. All the samples tested negative for non-*E. coli* enteropathogens. Coliform bacteria were found in 47 of the 49 (95.9%) samples from Guadalajara and in 10 of the 49 (20.4%) samples from Houston ( $P < 0.001$ ). The average coliform count was  $5.8 \times 10^4$  CFU/g (median  $0.7 \times 10^4$  CFU/gram) of dessert in Guadalajara compared with  $0.2 \times 10^4$  CFU/g (median of 0) of dessert in Houston ( $P < 0.0001$ ; Table 1).

*Escherichia coli* were found in 6/49 (12%) desserts from Guadalajara compared with 0/49 (0%) from Houston ( $P = 0.02$ ). ETEC was detected in four Mexico desserts: one dessert was positive for both a heat stable toxin-producing strain (ST-ETEC) and a heat labile toxin-producing strain (LT-ETEC) and the three others were positive for ST-ETEC strains. EAEC was identified in one dessert from Mexico. The sixth *E. coli* strain from Mexico was not identified as ETEC or EAEC, but was not further categorized to assure it was non-diarrheagenic.

Among the various types of desserts studied in Mexico including cream-filled, those topped with frosting, and desserts with ice cream, items with ice cream were found to be most frequently contaminated with *E. coli* compared to the desserts without ice cream ( $P = 0.007$ ). Four of the six dessert samples positive for *E. coli* contained ice cream. Additionally,

\* Address correspondence to Herbert L. DuPont, 1200 Herman Pressler, Suite 733, Houston, TX 77030. E-mail: Herbert.L.Dupont@uth.tmc.edu

TABLE 1

Identification of coliforms, *E. coli*, and diarrheagenic *E. coli* in desserts from public restaurants in Guadalajara, Jalisco, Mexico and Houston, Texas

	No. of samples with coliforms	Concentration of coliforms (CFU/g)		No. of samples with <i>E. coli</i>	Concentration of <i>E. coli</i> (CFU/g)		Presence of ETEC§	Presence of EAEC¶
		Mean	Median*		Mean	Median†		
Guadalajara, Mexico	47/49	$5.8 \times 10^4$	$0.7 \times 10^4$	6/49	$0.5 \times 10^4$	$0.3 \times 10^4$	4/6	1/6
Houston, TX	10/49	$0.2 \times 10^4$	0	0/49	0	0	0	0
<i>P</i> value	<0.001	NA‡	<0.0001	0.02	NA	0.003	NA	NA

\* Range: Guadalajara, Mexico:  $0-30.3 \times 10^4$ ; Houston, TX:  $0-0.32 \times 10^4$ .

† Range: Guadalajara, Mexico:  $0.05 \times 10^4-1.2 \times 10^4$ ; Houston, TX: 0-0.

‡ ETEC = enterotoxigenic *E. coli*.

§ EAEC = enteroaggregative *E. coli*.

¶ NA = not applicable.

desserts were also classified as being served hot, cold, or room temperature based on the temperature when served. The temperature differences were minimal and did not correlate with coliform or *E. coli* counts.

Food has been shown to be the principal source of bacterial enteropathogens causing travelers' diarrhea.<sup>7-9</sup> General recommendations given to travelers to high-risk areas include eating only cooked foods served steaming hot or those that can be peeled. Information regarding the safety of desserts is limited. A study among US military personnel in Alexandria, Egypt, found that consuming any meal that included meats, desserts, and buffet items was an independent risk factor for the development of traveler's diarrhea.<sup>10</sup> Sutherland and Limond<sup>11</sup> showed *in vitro* that very high sugar levels inhibited toxin production by *Bacillus cereus*, a well-established food-borne pathogen. This study provides evidence that the sugar content of most desserts fails to provide important protection against contamination by fecal bacteria. In this study, desserts from Mexico were frequently contaminated with coliforms, *E. coli*, and the two principal causes of travelers' diarrhea: ETEC and EAEC. The level of coliform and *E. coli* contamination of desserts samples from Houston was lower than that seen in Guadalajara, and the diarrhea-causing ETEC and EAEC were not found in Houston foods.

International guidelines for the microbiological quality of processed and ready to eat foods consider unsatisfactory for human consumption those containing  $\geq 10^4$  CFU/g of coliforms or  $\geq 10^2$  CFU/g of *E. coli*.<sup>3</sup> The average number of coliforms in desserts obtained from Guadalajara restaurants was  $5.8 \times 10^4$ , with average *E. coli* concentration of  $0.5 \times 10^4$  CFU/g, both in the unsafe range according to published microbial quality standards.<sup>3</sup>

A potentially important finding of this study was the common occurrence of microbial contamination of ice cream served in Guadalajara restaurants. A previous study in Cambodia and a second one in Costa Rica found coliform contamination of ice cream in 30% and 27-51% of samples, respectively.<sup>12,13</sup> In a third study in Guatemala, Peace Corp volunteers with <6 months of residence experienced a relative risk of 2.67 for development of diarrhea after eating ice cream.<sup>14</sup> This study focused on desserts served at restaurants, and we did not study fast-serve, popular ice cream parlors. Although unproven, it may be that improved ice cream quality would be seen in settings with high-volume sales.

In this study, we found desserts served at popular restaurants in one large Mexican city to commonly be contaminated with fecal organisms and enteric pathogens. We have previously found that ETEC can be found in 5-9% and EAEC

in 44% of red and green hot sauces found commonly on the tabletop in Guadalajara restaurants.<sup>8</sup>

Assuming our findings in Guadalajara are not unique, we conclude that desserts served in popular restaurants in developing countries may be considered to have risk for development of travelers' diarrhea. As a general rule, we now recommend that travelers not obtain desserts from public restaurants during travel to developing regions. We especially caution against the consumption of ice cream served at general restaurants in tropical and subtropical areas.

Received June 25, 2008. Accepted for publication October 3, 2008.

Financial support: This study was funded with discretionary funds from the University of Texas Health Science Center at Houston.

Authors' addresses: Karen J. Vigil and Charles Ericsson, The University of Texas Health Science Center at Houston Medical School, 6431 Fannin, MSB 2.112, Houston, TX 77030, Tel: 713-500-6765, Fax: 713-500-5495, E-mails: Karen.J.Vigil@uth.tmc.edu and Charles.D.Ericsson@uth.tmc.edu. Zhi-Dong Jiang, Thushara Galbadage, Eric L. Brown, and Hoonmo Koo, 1200 Herman Pressler Dr., RAS-E701, Houston, TX 77030, Tel: 713-500-9370, Fax: 713-500-9359, E-mails: Zhi-Dong.Jiang@uth.tmc.edu, Don.T.Galbadage@uth.tmc.edu, Eric.L.Brown@uth.tmc.edu, and Hoonmo.Koo@uth.tmc.edu. Jaclyn J. Chen and Kathryn L. Palumbo, The University of Texas Health Science Center at Houston Medical School, 6431 Fannin, JLL-304, Houston, TX 77030, Tel: 713-500-5140, Fax: 713-500-0603, E-mails: Jaclyn.J.Chen@uth.tmc.edu and Kathryn.L.Palumbo@uth.tmc.edu. Jing Yiang and Javier A. Adachi, 1515 Holcombe Blvd., Unit 1460, Houston, TX 77030, Tel: 713-794-4774, Fax: 713-794-4351, E-mails: yijiang@mdanderson.org and jaadachi@mdanderson.org. Margaret W. DuPont and Herbert L. DuPont, 1200 Herman Pressler, RAS-E733, Houston, TX 77030, Tel: 713-500-9366, Fax: 713-500-9359, E-mails: Mwdupont@swbell.net and Herbert.L.Dupont@uth.tmc.edu.

## REFERENCES

1. Von Sonnenburg F, Tornieporth N, Waijaki P, Lowe B, Perushi LF, DuPont HL, Mathewson JJ, Stephen R, 2000. Risk and aetiology of diarrhea at various tourist destinations. *Lancet* 356: 133-134.
2. Kozicki M, Steffen R, Schar M, 1985. "Boil it, cook it, peel it or forget it": does this rule prevent travellers' diarrhoea? *Int J Epidemiol* 14: 169-172.
3. Gilbert RJ, De Louvois J, Donovan T, Little C, Nye K, Ribeiro CD, Richards J, Roberts D, Bolton FJ, 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Commun Dis Public Health* 3: 163-167.
4. Jiang Z-D, Lowe B, Verenkar MP, Ashley D, Steffen R, Tornieporth N, von Sonnenburg F, Waijaki P, DuPont HL, 2000. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), and Jamaica (Montego Bay). *J Infect Dis* 185: 497-502.

5. Caeiro J-P, Estrada-Garcia MT, Jiang Z-D, Mathewson JJ, Adachi JA, Steffen R, DuPont HL, 1999. Improved detection of enterotoxigenic *Escherichia coli* among patients with travelers' diarrhea, by use of the polymerase chain reaction technique. *J Infect Dis* 180: 2053-2055.
6. Donnenberg MS, Nataro JP, 1995. Methods for studying adhesion of diarrheagenic *Escherichia coli*. *Methods Enzymol* 253: 324-336.
7. Wood LV, Ferguson LE, Hogan P, Thurman D, Morgan DR, DuPont HL, Ericsson CD, 1983. Incidence of bacterial enteropathogens in foods from Mexico. *Appl Environ Microbiol* 46: 328-332.
8. Adachi JA, Mathewson JJ, Jiang ZD, Ericsson CE, DuPont HL, 2002. Enteric Pathogens in Mexican Sauces of Popular Restaurants in Guadalajara, Mexico and Houston, Texas. *Ann Intern Med* 136: 884-887.
9. Tjoa WS, DuPont HL, Sullivan P, Pickering LK, Holguin AH, Olarte J, Evans DG, Evans DJ Jr, 1977. Location of food consumption and travelers' diarrhea. *Am J Epidemiol* 106: 61-66.
10. Haberberger RL, Scott DA, Thornton SA, Hyams KC, 1994. Diarrheal disease aboard a U.S. Navy ship after a brief port visit to a high risk area. *Mil Med* 159: 445-448.
11. Sutherland AD, Limond AM, 1993. Influence of pH and sugars on the growth and production of diarrhoeagenic toxin by *Bacillus cereus*. *J Dairy Res* 60: 575-580.
12. Windrantz P, Arias ML, 2000. Evaluation of the bacteriological quality of ice-cream sold at San Jose, Costa Rica. *Arch Latinoam Nutr* 50: 301-303.
13. Krui SL, Soares JL, Ping S, Sante Marie FF, 2001. Microbiological quality of "Ice, ice cream, sorbet" sold on the streets of Phnom Penh; April 1996-April 1997. *Bull Soc Pathol Exot* 94: 411-414.
14. Herwaldt BL, de Arroyave KR, Roberts JM, Juranek DD, 2000. A multiyear prospective study of the risk factors for and incidence of diarrheal illness in a cohort of Peace Corps volunteers in Guatemala. *Ann Intern Med* 132: 982-988.