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Real-Time PCR Detection of *Salmonella* in Suspect Foods from a Gastroenteritis Outbreak in Kerr County, Texas

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In June 2001, an outbreak of acute gastroenteritis among 109 attendees of a church picnic in Kerr County, Texas, was reported. A 5′-nuclease PCR assay was used to screen for *Salmonella* in nine food items from the buffet line. Barbeque chicken B tested positive for *Salmonella*, and no amplification was detected in the remaining food items. These PCR findings were consistent with culture results and were confirmed by direct nucleotide sequencing. *Salmonella enterica* serotype Panama was cultured from both food and patient stool samples.

Salmonella, one of the most common causes of food-borne disease outbreaks, is responsible for an estimated 1.4 million annual cases in the United States (data available at http/www.cdc.gov). Based on these statistics, a fluorogenic 5′-nuclease real-time PCR assay for the rapid detection of *Salmonella* spp. was developed by the U.S. Air Force as a public health measure for military personnel stationed worldwide. The primers and probe amplify and detect a 102-bp fragment of *invA*, a highly conserved gene present in almost all *Salmonella* serotypes (1, 3, 4, 9).

A detailed description of the development of this assay is to be reported elsewhere (L. T. Daum et al., unpublished data). In brief, the specificity of this PCR assay was evaluated against 111 culture-grown *Salmonella* serotypes and cross-tested against a reference panel of 37 related bacterial strains and human genomic DNA. Our assay was shown to detect all 111 *Salmonella* strains, and no nonspecific amplification was observed in a cross-reaction panel. Sensitivity was determined by real-time PCR amplification of serial 10-fold dilutions of genomic *Salmonella* DNA. Amplification of target sequences was consistently detected in samples containing less than 100 fg of genomic *Salmonella* DNA. Recently, a suspected outbreak of salmonellosis in central Texas afforded an opportunity to use this PCR assay to determine the possible presence of *Salmonella* contamination in various food items from a dinner buffet.

In June 2001, the Air Force Institute for Environmental Safety and Occupational Health Risk Analysis Epidemiological Surveillance Division at Brooks Air Force Base (AFB) was notified by the Texas Department of Health (TDH) of an outbreak of acute gastroenteritis among attendees of a buffet-style picnic at a church camp social in Kerr County, Texas. TDH officials conducted a formal investigation into the incident (10). Among the estimated 277 attendees (54% male, 46% female), 109 developed case-defined illness consistent with salmonellosis according to Centers for Disease Control and Prevention (CDC, Atlanta, Ga.) criteria (http/www.cdc.gov). The median incubation period was 16 h (range, 7 to 96 h). The predominant symptoms were diarrhea (95%), fever (90%), nausea (84%), abdominal cramps (83%), and vomiting (61%). Sixty individuals were treated and released by local hospitals, and nine were admitted overnight. A detailed epidemiological analysis of this outbreak has recently been reported by the TDH and is available online (10).

For confirmatory culture analysis at Brooks AFB, food items were homogenized for 1 min in 20 ml of brain heart infusion (Remel Microbiology Products, Lenexa, Kans.). A total of 0.5 ml of hemolysate was inoculated onto blood agar and xylose, lysine, and deoxycholate plates (Remel) and incubated at 37°C for 24 h. *Salmonella*-positive cultures were confirmed by biochemical analysis with a Vitek instrument (Vitek Systems, Hazelwood, Mo.). Additional samples were submitted to the TDH Bureau of Laboratories, where they were tested for common enteric bacterial pathogens and enteric viral species.

For PCR, samples were collected from nine food items served from the buffet dinner. Nucleic acids were extracted directly from food samples and subjected to fluorescent real-time PCR using a "ruggedized" automated pathogen identification device (RAPID; Idaho Technologies, Salt Lake City, Utah). For DNA isolation, two protocols were tested, the Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.) and the capture disk DNA isolation kit (Gentra Systems). Of the two methods for DNA extraction, a modified version of the capture disk protocol was utilized because it was simple to use and highly reliable with food samples (7). Food items were homogenized in sterile water for 1 min, and then 200 μl of homogenized suspension was transferred into a spin tube containing a capture disk. Following a 10-min incubation period at room temperature, the tube was centrifuged at 12,000 × *g* for 20 s. A total of 200 μl of DNA purification solution was added to the spin tube, which was then incubated

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TABLE 1. Fluorogenic primers and probe used for amplification of a 102-bp region of the *invA* gene of *Salmonella* spp.

Description	Sequence[a]	T_m (°C)
Sal-F (forward primer)	5′-GCGTTCTGAACCTTTGGTAATAA-3′	57
Sal-R (reverse primer)	5′-CGTTCGGGCAATTCGTTA-3′	57
Sal-TM (probe)	5′-FAM-TGGCGGTGGGTTTTGTTGTCTTCT-TAMRA-3′	63

[a] FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

for 1 min at room temperature and centrifuged at 12,000 × *g* for 20 s. The DNA purification step was repeated two additional times. With a sterile pipette tip, the capture disk was removed from the column and added directly to a PCR capillary tube containing master mix. The PCR master mix consisted of 18 μl of 1× hybridization buffer (LightCycler DNA master hybridization probes; Roche Molecular, Indianapolis, Ind.), 5 mM $MgCl_2$, 500 nM (each) forward and reverse primers, and 100 nM probe. The *Salmonella invA* probe was labeled with 6-carboxyfluorescein (reporter dye) and 6-carboxytetramethylrhodamine (quencher dye). The primers and probe set used in this investigation are characterized in Table 1. DNA from a culture-grown stock of *Salmonella enterica* was used as a positive control. PCR was performed using the RAPID (Idaho Technologies). The PCR cycling consisted of a 1-min denaturation at 94°C followed by 40 cycles of 94°C for 0 s and 60°C for 20 s. The amplification of a 102-bp fragment from the *invA* gene was detected by monitoring the increase in fluorescence from the dye-labeled *Salmonella*-specific probe. If the probe was cleaved, the reporter and quencher dyes were released, causing an increase in reporter dye fluorescence. The threshold cycle (C_T) is defined as the PCR cycle where a significant increase in fluorescence was first observed (according to the LightCycler owner's manual [Roche Molecular], version 1.2). This value is normalized against background fluorescence.

For each of the nine food items tested, two samples, designated A and B, were obtained from different areas of the food container and used directly for PCR. Barbecue chicken B tested positive for *Salmonella* (C_T, 23.54), while no amplification for *Salmonella* DNA was detected in the remaining samples (Table 2). Another food item, beans, was also served at the buffet dinner. Unfortunately, this food item had been discarded and was not among the samples provided to the U.S Air Force or the TDH. To confirm the presence of *Salmonella*, a PCR product originating from chicken B was subjected to direct nucleotide sequencing with a PE 377 automated sequencer with the Big Dye chemistry set (PE Biosystems, Foster City, Calif.) according to manufacturer recommendations. A 64-bp portion of our 102-bp amplicon from chicken B was sequenced and determined to be 100% (64 of 64) homologous to the *invA* gene of *S. enterica*.

By using traditional culture methods, the TDH isolated *S. enterica* serotype Panama from chicken B and from corn on the cob (Table 2). Stool cultures collected from 1 employee and 24 dinner attendees were also culture positive for *S. enterica* serotype Panama. From the food samples analyzed by Brooks AFB, chicken B was the only food item that tested positive by both culture and real-time fluorescent PCR (Table 2).

The TDH conducted a comprehensive outbreak investigation, which included an on-site kitchen inspection, the collection of food samples, and interviews with patients and kitchen staff. The dinner was eaten by a total of 277 people; of these, 237 (86%) were interviewed by phone, and 3-day illness histories were obtained. Of these 237, 109 individuals met case patient criteria. A case patient was one who ate the supper and either exhibited three of the most frequently reported symptoms (i.e., diarrhea, fever, nausea, abdominal cramps, or vomiting) or had a stool culture positive for *Salmonell*a serotype Panama (12). Using the software package EpiInfo (CDC), an epidemiological analysis was performed by generating odds ratio (OR) correlations comparing food items ingested with the incidence of disease. Beans (OR = 27.86) and chicken (OR = 2.20) were the only food items with ORs that were statistically significant at the 95% confidence level, indicating these items as the probable culprits in this food-borne outbreak. Only five individuals who reported eating chicken and beans remained asymptomatic.

Although the TDH isolated *Salmonella* from corn on the cob by culture, the sample submitted to Brooks AFB was negative by both culture and real-time PCR. According to epidemiological analysis, the OR for the corn on the cob was not statistically significant (OR = 1.26). These epidemiological data, along with culture and real-time PCR results from Brooks AFB, suggest the possibility of low-level cross contamination of this food item during preparation, or at the buffet during sample collection. Because raw chicken is often contaminated with *Salmonella*, it is most likely that uncooked or partially cooked chicken was the original *Salmonella* source and that the beans

TABLE 2. Food items tested for *Salmonella* by real-time PCR and culture methods

Sample	Result[a] of:		
	Real-time PCR (C_T)	Culture	
		BAFB	TDH
Food items			
Sausage A and B	N	N	N
Corn A and B	N	N	P
Potato salad A and B	N	N	N
Barbecue chicken A	N	N	N
Barbecue chicken B	P (23.54)	P	P
Cole slaw A and B	N	N	N
Pasta salad A and B	N	N	N
Brisket A	N	N	N
Brownie A	N	N	N
Corn muffin A and B	N	N	N
PCR controls			
S. enterica (1 ng/μl)	P (16.63)		
No template (negative)	N		

[a] BAFB, Brooks AFB; N, negative; P, positive.

and corn on the cob became contaminated by unsanitary cooking practices.

Many studies have used fluorogenic PCR for the rapid detection of *Salmonella* spp. from culture and directly from food items (2, 5, 6, 8). Currently, the detection of *Salmonella* by local health agencies is primarily accomplished through traditional culturing techniques that can take up 2 to 6 days to complete and may be too time-consuming in cases where rapid pathogen identification is critical. The fluorogenic TaqMan PCR assay described here confirmed the presence of *Salmonella* directly from chicken in less than 3 h. Our study, therefore, illustrates the feasibility of using these assays during real-world outbreaks.

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