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Research Note

Detection of *Salmonella enterica* Somatic Groups C1 and E1 by PCR–Enzyme-Linked Immunosorbent Assay

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ABSTRACT

Objectives of this study were to develop a PCR-based enzyme-linked immunosorbent assay (PCR-ELISA) for identification of *Salmonella enterica* somatic groups C1 and E1 and to evaluate this procedure along with a PCR-ELISA procedure for *S. enterica* somatic groups B, C2, and D in a masked study. Primers were selected from the *rfb* gene cluster, which is responsible for biosynthesis of O antigens of *Salmonella* lipopolysaccharide. Previously serogrouped *Salmonella* isolates (n = 169) were used to determine the sensitivity and specificity of the PCR-ELISA procedure. DNA from all isolates was amplified using the PCR procedure for selected somatic groups and amplified products were visualized on agarose gels, as well as subjected to the ELISA procedure. The PCR-ELISA technique correctly identified 97% of somatic group C1 and 87% of somatic group E1. The sensitivity of this procedure to correctly identify *S. enterica* somatic group C1 was 97% and 88% for somatic group E1. The specificity was 98% for both somatic groups C1 and E1. The PCR-ELISA techniques correctly identified 93% of *Salmonella* isolates belonging to somatic groups B, C1, C2, D, and E1. The overall sensitivity of this procedure to correctly identify *S. enterica* somatic groups was 96% and the specificity was 98%. Ninety-one percent of somatic group D, 92% of somatic group B, and 97% of somatic group C2 were identified correctly with this procedure. Results of this study indicate that the PCR-ELISA procedure is a rapid and accurate method for serogrouping *Salmonella* isolates. Utilization of the PCR-ELISA procedure for *Salmonella* serogrouping would aide in identification, surveillance, prevention, and control of *Salmonella.*

Salmonella is an important foodborne pathogen that is responsible for serious cases of foodborne illness. *Salmonella* can be transmitted in a wide variety of agricultural products and processed foods. Foods of animal origin such as beef, pork, chicken, eggs, and milk have been shown to carry these pathogens. In particular, fecal shedding of *Salmonella enterica* serogroup E1 has been reported to represent 15 to 40% of all *Salmonella* serogroups isolated from beef and dairy cows, and serogroup C1 has been reported to represent 20 to 35% of all *Salmonella* serogroups isolated from fecal samples of beef and dairy cows *(2, 4, 14).* Identification of pathogenic microorganisms, including *Salmonella,* is important for surveillance, prevention, and control of foodborne diseases *(1).* An accurate and rapid procedure for identification of *Salmonella* is needed to identify sources, reservoirs, and transfer of these foodborne pathogens through the food chain.

Current available screening tests only provide presumptive identification of *Salmonella* as a group without identification of serogroups. Negative results are considered definitive, but positive results must be confirmed by conventional methods and serology *(5).* In *Salmonella,* the *rfb* gene clusters are responsible for biosynthesis of the O antigens of *Salmonella* lipopolysaccharide *(12, 15).* Differentiation of *Salmonella* serogroups is based on variability of O antigen structures, which is apparent in the arrangement and types of sugars present. This highly polymorphic *rfb* gene cluster has been targeted as a molecular marker for the organism for detection of *Salmonella* somatic groups *(8, 9, 10).* Objectives of this study were to develop a PCR-based enzyme-linked immunosorbent assay (PCR-ELISA) for identification of *S. enterica* somatic groups C1 and E1 and to evaluate this procedure along with a PCR-ELISA described by Luk et al. *(8, 9, 10)* to identify *Salmonella* somatic groups B, C2, and D.

MATERIALS AND METHODS

Bacterial strains. Eight American Type Culture Collection (ATCC, Rockville, Md.) *Salmonella* strains used as control organisms included the following *S. enterica* subsp. *enterica* serotypes: Paratyphi A, Typhimurium, Choleraesuis, Enteritidis, Newport, Anatum, Worthington, and Montevideo. These strains represent the following serogroups: A, B, C1, D1, C2, E1, G2, and C1, respectively. In addition, eight strains from the *Enterobacteriaceae* group were included to determine potential cross-reactivity. These included *Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, Serratia marcescens,* and *Shigella flexneri.*

Salmonella isolates (n = 100) serogrouped at the U.S. Department of Agriculture Animal and Plant Health Inspection Ser-

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TABLE 1. *Oligonucleotide primers used for* Salmonella *PCR-ELISA assay*

Oligonucleotide primers	Somatic group specificity	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
rfb(B)F	B	5′-GAGAATATGTAATTGTCAG-3′	851	Luk et al. *(9)*
rfb(B)R		5′-GTAACCGTTTCAGTAGTTC-3′		
rfb(C2)F	C2	5′-ATGCTTGATGTGAATAAG-3′	795	Luk et al. *(9)*
rfb(C2)R		5′-CTAATCGAGTCAAGAAAG-3′		
rfb(C1)F	C1	5′-GGCGCTGATTTAACAGGTG-3′	781	This study
rfb(C1)R		5′-CATAAGCACAGTCACAACCTGG-3′		
rfb(D)F	D	5′-AGTCACGACTTACATCCTAC-3′	703	Luk et al. *(9)*
rfb(D)R		5′-ACCTGCTATATCAGCACAAC-3′		
rfb(E1)F	E1	5′-ATCGGCAAGTGTATATCTAACG-3′	189	This study
rfb(E1)R		5′-CCAACCTCAGTAGCAACAAC-3′		

vices (USDA-APHIS, Ames, Iowa) and at Washington State University (Pullman, Wash., n = 69) were evaluated by the PCR-ELISA procedure. The majority of *Salmonella* isolates (80%) were submitted from bovine or swine samples, with the remaining isolates submitted from domestic farm animals, exotic animals, or reptiles. All isolates were stored at −80°C until the PCR-ELISA analysis was performed.

DNA isolation. All *Salmonella* strains were first streaked on XLD agar (Becton Dickinson and Company, Franklin Lakes, N.J.), and one colony was selected for DNA isolation by the InstaGene matrix (Bio-Rad, Hercules, Calif.) following manufacturers' directions as described by Gillespie et al. *(6)*. For PCR analysis, 10 μl of the supernatant were used.

PCR. Primers for PCR to detect *Salmonella* somatic group C1 were designed (Gene Runner v3.05, Hastings Software, Inc., New York) on the basis of the sequence of *S. enterica* group C1 *rfb* gene cluster *(7)*. Primers for PCR to detect *Salmonella* somatic group E1 were designed (Gene Runner v3.05) on the basis of the sequence of *S. enterica* group E1 *rfb* gene cluster *(13)*. The PCR primers for *Salmonella* somatic groups B, C2, and D were reported previously *(8, 9, 10)*. Primer sequences used in the PCR-ELISA are in Table 1. A separate PCR was performed for each specific serogroup of *Salmonella*, with positive and negative controls included in each PCR run. Positive controls included DNA from *Salmonella* ATCC strains and negative controls included replacement of DNA with 10-μl sterile H_2O.

Primers were synthesized (IDT, Coralville, Iowa), with the 5′ end labeled with biotin. DNA amplification was performed following the protocol of Luk et al. *(8, 10)* with modifications. Conditions for *Salmonella* somatic groups B, C1, C2, and D were as follows: 10 μl DNA; 0.2 μg of each biotin-labeled primer; 200 μM each of dATP, dCTP, and dGTP; 150 μM of dTTP and 50 μM digoxigenin-11′-dUTP (Boehringer Mannheim, Indianapolis, Ind.), 10 U *Taq* polymerase (Promega, San Diego, Calif.); 10 μl 10× magnesium-free thermophilic buffer [500 mM KCl, 100 mM Tris-HCl (pH 9.0), and 1% Triton X-100]; 8 μl 25 mM $MgCl_2$; and sterile H_2O to bring the total volume to 100 μl. Following an initial denaturation step at 94.5°C for 5 min, parameters for the thermocycler (iCycler, Bio-Rad, Hercules, Calif.) were set at 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s. DNA was amplified for 30 cycles, followed by an extension step of 72°C for 150 s. Conditions for *Salmonella* somatic groups E1 were as described above with the following changes: 0.1 μg of each biotin-labeled primer, 2.5 U *Taq* polymerase (Promega), 6 μl 25 mM $MgCl_2$, and an annealing temperature of 54°C for 30 s. Detection of PCR products by gel electrophoresis and photography of agarose gels were as described by Gillespie et al. *(6)*.

ELISA. To detect PCR products by ELISA, the protocol described by Luk et al. *(8, 10)* was used for all *S. enterica* somatic groups tested. Briefly, 96-well microtiter plates (Costar, Corning Inc., Corning, N.Y.) were coated with streptavidin (5 mg/ml, Sigma-Aldrich Corp., St. Louis, Mo.), and unsaturated binding sites were blocked with bovine serum albumin (Sigma-Aldrich Corp.). Samples of the diluted PCR product (10 μl sample and 90 μl PBS, pH 7.4) were added in triplicate to streptavidin-coated wells and incubated at 37°C for 1 h. Positive and negative PCR controls were run on each plate, as well as a reagent blank. Anti-digoxigenin Fab-alkaline phosphate conjugate (Boehringer Mannheim) was added to each well and incubated at 37°C for 1 h. After washing, 100 μl of substrate solution (*p*-nitrophenylphosphate; Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was added, and color was developed at 37°C for 30 min. ELISA absorbance was measured at 405 nm with a BIO-TEK microplate reader (BIO-TEK Instruments, Inc., Winooski, Vt.).

Determination of sensitivity and specificity. *Salmonella* isolates (n = 169) were evaluated in a masked study to determine sensitivity and specificity of PCR-ELISA for somatic groups C1 and E1, as well as other *S. enterica* somatic groups (B, C2, and D). The following formulas were used *(11)*: sensitivity % = true positive samples/(true positive samples + false negative samples) × 100 and specificity % = true negative samples/(true negative samples + false positive samples) × 100.

RESULTS AND DISCUSSION

Salmonella (n = 169) isolated primarily from bovine and swine samples submitted to USDA-APHIS (n = 100) from various states and serogrouped at USDA-APHIS by the method described by Ewing *(3)*, and *Salmonella* isolates previously serogrouped and obtained from Washington State University (n = 69) were evaluated by PCR-ELISA to determine sensitivity and specificity of the procedure. The PCR-ELISA technique identified correctly 97% (34 of 35) of somatic group C1 and 87% (27 of 33) of somatic group E1 (Table 2). The sensitivity of this procedure to correctly identify *S. enterica* somatic group C1 was 97% and 88% for somatic group E1. The specificity was 98% for both somatic groups C1 and E1.

Combining the PCR-ELISA developed in this study for

TABLE 2. *Results of PCR-ELISA on previously identified* Salmonella enterica *somatic groups B, C1, C2, D, and E1*

Serogroup	Total isolates (*n*)	Correct identification (*n*)	False positive (*n*)	False negative (*n*)	Correct identification (%)
D	33	30	0	3	91
B	26	24	1	2	92
C1	35	34	2	1	97
C2	32	31	1	1	97
E1	31	27	2	4	87
Total[a]	157	146	6	11	93

[a] Twelve additional *Salmonella* isolates were tested with the PCR-ELISA. Five somatic groups belonged to G2 and seven somatic groups belonged to K; none were positive with PCR-ELISA for somatic groups B, C1, C2, D, or E.

S. enterica somatic groups C1 and E1 with the PCR-ELISA developed by Luk et al. *(8, 10)* for *S. enterica* somatic groups B, C2, and D, 93% (146 of 157) of *Salmonella* isolates were identified correctly. A sensitivity of 96% and a specificity of 98% were determined for the PCR-ELISA to identify all *Salmonella* somatic groups B, C1, C2, D, and E1. The PCR-ELISA procedure identified correctly 30 of 33 isolates as somatic group D (Table 2). Twenty-four of 26 isolates were identified as somatic group B (Table 2). One somatic group B isolate was identified as C2 and two isolates were identified as both B and E1 by this method but were previously typed as B. Thirty-four of 35 isolates were identified correctly as somatic group C1. Two isolates were positive for somatic group C1, but were typed previously as somatic group E1 (Table 2). The PCR-ELISA correctly identified 31 of 32 *Salmonella* somatic group C2 isolates (Table 2). Twenty-seven of 31 isolates were identified as *Salmonella* somatic group E1, 2 somatic group B isolates were identified as E1 by this method (Table 2). The remaining isolates belonged to somatic groups G2 ($n = 5$) and K ($n = 7$), and no PCR-amplified fragments or positive ELISA readings were observed with these isolates with primers specific for somatic groups B, C1, C2, D, or E1. All *Salmonella* isolates that were identified incorrectly by PCR-ELISA were submitted to USDA-APHIS for confirmation. Results of the second tests confirmed the previous serogroup by USDA-APHIS or Washington State University.

Distinct PCR products were observed with primer pairs for specific somatic groups by agarose gel electrophoresis with the eight *Salmonella* ATCC strains. Fragment sizes included: 781, 189, 795, 703, and 851 base pairs for somatic groups C1, E1, C2, D1, and B, respectively (Table 1). Eight strains from the *Enterobacteriaceae* group were included to determine potential cross-reactivity; however, no cross-reactivity was observed with any of the primer pairs for specific somatic groups with any of the *Enterobacteriaceae* strains tested.

Results of this study indicate that primers for *Salmonella* somatic groups B, C1, C2, D, and E1 are specific and cross-reactivity does not occur, except for somatic group A cross-reacting with somatic group D. Cross-reactivity of somatic group A with D primer pairs was also noted previously *(9)*. *Salmonella* serogroups A and D differ only at one nucleotide base between the two *rfb*E genes; therefore, it is very difficult to design primers to distinguish between these two serogroups *(9)*. The PCR-ELISA procedure correctly identified 146 of 157 *Salmonella* isolates as serogroups B, C1, C2, D, or E1. This technique correctly identified 97% of *Salmonella* somatic groups C1 and C2, 92% of group B, 91% of group D, and 87% of group E1. This resulted in an overall sensitivity of 96% and a specificity of 98%.

This research expanded the technique of Luk et al. *(8, 10)* and developed primers specific for *Salmonella* somatic group C1 and E1 that could be used in a PCR-ELISA assay. With the addition of these somatic groups, a total of five different *S. enterica* somatic groups can now be identified using the PCR-ELISA procedure. This method also allows simultaneous testing of a large number of suspected *Salmonella* samples. Further development of this procedure to include detection of *Salmonella* pathogens in fecal or food samples is needed to facilitate the identification, prevention, and control of *Salmonella* through the food chain.

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