PCR Detection and Microbiological Isolation of *Salmonella* spp. from Fresh Beef and Cantaloupes


**ABSTRACT:** Species belonging to the genus *Salmonella* are an important cause of enteric fevers, gastroenteritis, and septicaemia, and the pathogens are commonly transmitted through contaminated food. In this study, polymerase chain reaction (PCR) amplification of a 287-bp region of the invA gene was compared to a microbiological technique to determine the presence of *Salmonella* in retail beef and in cantaloupe rinse samples. Both methods showed the same level of sensitivity, detecting 1 CFU/25 g of meat after enrichment for 24 h at 42 °C. The presence of *Salmonella* was determined in 50 commercial top sirloin beef samples that were not artificially inoculated. Three samples were positive by the microbiological method, and these samples and an additional sample were positive by the PCR. Both methods were also used to test surface rinses of cantaloupes collected from 4 farms in Nayarit, Mexico. *Salmonella* was detected by the microbiological method in 9 of 20 samples (45%), whereas the pathogen was detected by the PCR in 11 samples (55%). This study demonstrates the utility of the PCR targeting the invA gene to determine the presence of *Salmonella* spp. in beef and cantaloupe samples.

**Key words:** cantaloupe, detection, meat, PCR, *Salmonella*

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**Introduction**

Salmonellosis caused by species in the genus *Salmonella* was described in 1984 as a "new and significant threat to the public health" by the World Health Organization (FAO 1984), and *Salmonella* has remained a major foodborne pathogen associated with different types of food. Gutiérrez and others (2000) reported the isolation of *Salmonella* spp. in Mexico in 51% of fast food samples, 23% of processed meat products (ham, chorizo, and bacon), 22% of ground food samples (beef, chicken, fish), 3% of milk products, and in 1% of both fresh and powdered eggs. Salmonellosis outbreaks in the United States linked to the consumption of vegetables imported to the United States in 1999 showed that of 1003 analyzed samples, 35 (3.5%) tested positive for *Salmonella* spp. in Mexico in 51% of fast food samples, 23% of processed meat products (ham, chorizo, and bacon), 22% of ground food samples (beef, chicken, fish), 3% of milk products, and in 1% of both fresh and powdered eggs. Salmonellosis outbreaks in the United States linked to the consumption of cantaloupes implicated *Salmonella* serotypes Saphra and Poona as the causative agents from cantaloupes that originated from Mexico (Mohle and others 1999; CDC 2002). Analyses of fruits and vegetables imported to the United States in 1999 showed that of 1003 analyzed samples, 35 (3.5%) tested positive for *Salmonella* spp., and of these, 8 were cantaloupe samples (22.9%), indicating that cantaloupe was the 2nd most contaminated type of product after cilantro (FDA 2001). Currently in Mexico, the official procedure for detection of *Salmonella* spp. is a cultural method, and this procedure could take from 3 to 5 d for confirmation, which is a disadvantage when the results are needed promptly (SSA 1994; Peplow and others 1999). Molecular methods, such as the polymerase chain reaction (PCR), have shown high sensitivity and specificity for detecting target pathogens, including *Salmonella*, in different types of foods, and the time required to obtain results can be as short as 12 h (Ferretti and others 2001; Croci and others 2004). However, microbiological techniques are used as reference methods to demonstrate the efficacy and validity of new techniques (Fernandez 2000). The objective of this study was to compare the sensitivity of a PCR assay to a microbiological method and to evaluate the 2 methods for the detection of *Salmonella* spp. in naturally contaminated beef and cantaloupe rinse samples.

**Materials and Methods**

**Bacterial strain, growth conditions, and preparation of inoculum**

*S. Typhimurium* ATCC 13311 was grown on trypticase soy agar (TSA) (Becton Dickinson Co., Sparks, Md., U.S.A.) at 37 °C for 24 h. Afterward, 10 mL of physiological saline solution (0.85%) were added to the plate to obtain a homogeneous suspension of bacteria. An aliquot of the suspension was diluted to a concentration of the nr 5 tube of the MacFarland scale (1.5 x 10^8 CFU/mL). Ten-fold serial dilutions were prepared in saline solution to give suspensions containing 10^8 to 10^4 CFU/mL.

**Artificial inoculation of top sirloin meat samples**

The top sirloin meat samples were obtained on the same date from supermarkets in Monterrey, Nuevo León, Mexico. Meat samples were placed into a cooler (at 4 °C) and transported to the laboratory. The samples were immediately separately inoculated with the previously mentioned dilutions as described subsequently. Three sterile plastic bags (Whirl-Pak®, Nasco, Modesto, Calif., U.S.A.) with the capacity of 500 mL were labeled for each dilution, and 25 g of top sirloin meat were weighed and placed in each plastic bag with 225 mL sterile buffered peptone water (BPW) (Becton Dickinson and Co.). An uninoculated control sample was
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included to ensure that the meat was not naturally contaminated with Salmonella. One milliliter of each bacterial dilution (10^6 to 10^8 CFU/mL) was added to the corresponding plastic bag, which was then mixed by hand for 2 min followed by incubation at 35 °C for 24 h. The enrichment and microbiological analyses were performed using tetrathionate (TT) broth (Becton Dickinson Co.) according to the method described in the Microbiology Laboratory Guidebook (FSIS-USDA 2004). The experiments were performed in triplicate.

DNA extraction from inoculated top sirloin meat samples

Three milliliters from the Tetrathionate broth enrichments were used to form cell pellets by centrifuging at 3000 rpm for 5 min. DNA extraction from the cell pellets was performed using the CTAB (cetyl trimethyl ammonium bromide) method; however, the use of polyvinylpyrrolidone and β-mercaptoethanol was omitted (Doyle and Doyle 1987). The extracted DNA was stored at −20 °C.

PCR amplification conditions

Amplification of the target sequence was performed using a PCR Express thermal cycler (PCR Express; Thermo Hybaid, Middlesex, U.K.). The PCR mixture contained 25 pmoles of each of the primers targeting the invA gene (Rahn and others 1992), 200 μM of each of the 4 deoxynucleoside triphosphates (Bioline Inc., Randolph, Mass., U.S.A.), 1 mM MgCl₂, 1× Reaction Buffer (200 mM Tris-HCl pH 8, 500 mM KCl), 2.5 U of Taq DNA polymerase (Promega, Madison, Wis., U.S.A.), 100 ng of DNA template, and deionized water for a final volume of 25 μL. The reaction mixture was subjected to the following thermal cycling conditions: heat denaturation at 95 °C for 1 min, and then 35 cycles with heat denaturation at 95 °C for 30 s, primer annealing at 58 °C for 30 s, and DNA extension at 72 °C for 30 s. After the last cycle, samples were maintained at 72 °C for 10 min to complete synthesis of all strands. The PCR products were subjected to gel electrophoresis (1.5% agarose; Promega), and the amplification products or due to stressful storage conditions, which can damage or even kill the Salmonella. This in turn would affect the ability to detect the pathogen using the cultural method, since this method is dependent on growth of the cells. Figure 1 shows the

Table 1 — Meat samples positive for Salmonella spp., by both the cultural and PCR methods.

<table>
<thead>
<tr>
<th>Sample nr</th>
<th>Cultural method</th>
<th>PCR assay</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>−</td>
<td>+</td>
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<tr>
<td>33</td>
<td>+</td>
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<tr>
<td>41</td>
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PCR products obtained following amplification of the positive meat samples. Sample nr 12 (lane 3) shows a weak band. Thus, it is possible that there was less PCR product from this sample because there were a lower number of cells after enrichment compared to the other samples. This was likely the reason why the pathogen was not detected by plating. Although, the sensitivity of both the microbiological and PCR methods was the same (1 CFU/25 g) when meat samples were inoculated with nonstressed cells, in naturally contaminated samples, the cells may be stressed affecting their ability to grow as rapidly as nonstressed cells in the enrichment medium.

Cultural and PCR assays using cantaloupe rinse samples

The data in Table 2 show that in the 1st field, 4 positive samples were detected (quadrants I to III and V) by the microbiological method, and there were 4 positives from the same 4 quadrants by the PCR. However, for quadrants III and IV, the results of both methods differed. In the 2nd field, the results of both methods were the same, with positive results from samples in all 5 quadrants. In the 3rd and 4th fields, all results using the microbiological assay were negative, while using the PCR, results were positive from samples from the 3rd field from quadrants II and III and were negative from the 4th field from all 5 quadrants. With the exception of samples from quadrant III and IV from the 1st field where results differed, results of the PCR assay agreed with those of the cultural method, and in some cases the PCR assay was more sensitive for detection of Salmonella (quadrants II and III of the 3rd sampling). In summary, Salmonella spp. were detected by the microbiological method in 9 of 20 samples (45%), whereas the pathogen was detected by the PCR in 11 samples (55%). These results are partially in agreement with those of Espinoza-Medina and others (2006) who found that by 25.7% of samples from in-field cantaloupes were positive for Salmonella, whereas no positive samples were detected by the standard method.

The detection of this pathogen by the PCR was done from an enrichment culture; therefore, in addition to growth of Salmonella, the microflora from the melon samples also grew. Thus, the PCR was sensitive and specific, since Salmonella was detected in the presence of other microorganisms found in the melon production environment. Previous microbiological studies conducted in the Lagunera Region of Mexico (Froto and others 2004) found the presence of bacteria that belonged to the Enterobacteriaceae family and other microorganisms in the cuticle of melons, as well as plant pathogens such as Fusarium spp., Verticillium spp., and Rhizoctonia solani, and saprophytes such as Aspergillus spp., Rhizopus spp., and Penicillium spp. Also, human pathogens, including Clostridium botulinum, Listeria monocytogenes, Vibrio cholerae, Brucella melitensis, Salmonella Typhi, Salmonella Paratyphi, hepatitis A virus, Escherichia coli, and Shigella dysenteriae, were found. Most of these pathogens could be found in the soil coming from bovine and avian manure and from human feces (Froto and others 2004). The absence of the Salmonella invA sequence in other invasive bacteria such as Yersinia spp., Shigella spp., and enteroinvasive E. coli, which also have the capacity to invade epithelial cells, demonstrates the particular specificity and utility of this primer pair for detection of Salmonella spp. (Galán and Curtiss 1991). The invA gene has been used as the target in PCR assays mainly for detecting Salmonella in poultry, meats, and dairy products, and in vegetables and fruits (Guo and others 2000). The PCR results of the current study shown in Figure 2 indicate that the primers could be used for detection of Salmonella from cantaloupe surface washings and potentially in other types of fruits and vegetable samples, as well.

The differences in results from field to field may have been due to differences in levels of Salmonella contamination. This may have been influenced in part by changes in the environment, including loss of specific nutrients and fluctuations in humidity, temperature, and ultraviolet light, all of which could damage bacterial cells and...
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Although meat is usually not consumed raw, there is risk of Salmonella infection if the meat is improperly cooked, and there is also the possibility of cross contamination of Salmonella with foods that are consumed raw. Produce may become contaminated in the field through the use of contaminated irrigation water or manure or also from animals or inadequate worker hygiene. Therefore, the ability to rapidly detect Salmonella in meat, fruit, and other foods could lower the risk of contaminated food reaching the consumer. Use of a sensitive assay for detection of Salmonella in melons is also very important, since this food is eaten raw. The PCR assay evaluated in the current study could be used as a screening test, since results would be available in less time than with the cultural method. PCR-positive results could then be confirmed by the cultural method. Because the invA gene is present in pathogenic Salmonella serotypes, the PCR assay based on the primer pair targeting this gene could be applied for detection of Salmonella spp. that may be associated with particular food products, including poultry and food products that are consumed raw such as fruits and vegetables and/or ready-to-eat food. Further research will focus on validating the robustness of the Salmonella PCR assay in approved laboratories in Mexico for its use as a screening test using different types of samples, along with confirmation of the pathogen by the microbiological method. Additional studies to determine the most prevalent Salmonella serotypes found in beef and in cantaloupes and to determine the effect of environmental changes and the use of GAP on the prevalence of Salmonella in these foods are warranted.

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References


