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Research Note

Evaluation of Microbial Contamination of Tomatoes and Peppers at Retail Markets in Monterrey, Mexico

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ABSTRACT

The source of a large outbreak of foodborne disease related to *Salmonella*-contaminated jalapeño peppers has been traced to Nuevo Leon, Mexico. The objective of this work was to evaluate the microbiological quality of tomatoes and jalapeño peppers from markets and supermarkets from the metropolitan area of Monterrey, Nuevo León, Mexico. One hundred sixty samples (40 bola tomatoes, 40 saladette [Roma] tomatoes, 40 serrano peppers, and 40 jalapeño peppers) were purchased. Stems from peppers were removed and analyzed separately. Samples were analyzed for indicator organisms and *Salmonella,* following the Mexican Official Methods. The results showed that the presence of indicator organisms varied among samples and origins, and levels were relatively high in peppers (average 4.4 to 4.7 log CFU/g for total mesophilic, 3.25 to 3.73 log CFU/g for total coliforms, and 1.69 log CFU/g for fecal coliforms). Saladette tomatoes and serrano peppers showed the greatest microorganism levels (~1 log CFU/g higher) in comparison with the other varieties. Pepper stems typically had indicator microbial levels ~1 to 2 log CFU/g higher than levels in smooth flesh. Only one tomato and one jalapeño sample were positive for *Salmonella.* However, in the case of the pepper, the contamination was found in the stem. Although the microbiological quality of tomatoes and peppers sampled was similar to that found in markets from developed countries, the presence of pathogens causes a risk of infection for consumers.

Consumption of fresh produce has increased within the past decade, mainly because of awareness of the benefits of a healthy diet and the impact of governmental campaigns in several countries, such as the ''Five a Day'' and ''Nine a Day'' programs in the United Kingdom and the United States, and global efforts, such as those by the World Health Organization *(29)*. However, fresh vegetables can be contaminated with pathogens from animal and human sources during growth, harvest, transportation, and further handling *(11, 20)*. Contaminated soil, animal fecal droppings, poor worker hygiene, inadequately composted or raw animal manure or sewage, poor water quality for irrigation or washing, etc., contribute to the possible contamination of produce with microbial pathogens; in general, lapses in safety practices can produce product contamination *(3, 4, 13, 20, 24)*.

Due to these contributing factors, produce has been involved in an increasing number of foodborne disease outbreaks *(18)*. This increase in outbreaks can also be attributed to changing consumer habits, new methodologies to detect microorganisms, improved surveillance, and the globalization of the produce trade *(20)*.

The epidemiology of foodborne disease has changed rapidly over the last decades; and some major human pathogens, such as *Escherichia coli* O157:H7, *Salmonella,* and *Campylobacter,* have been recognized to be spread from animal reservoirs *(15)*. Fresh vegetables have emerged as vehicles for the transmission of these infectious diseases *(11)*. Leafy greens, tomatoes, cucurbits, peppers, and nuts have been among the foods commonly linked to outbreaks of gastrointestinal illnesses caused by *E. coli* O157:H7 and *Salmonella (17)*. *Salmonella* spp. are the most commonly identified etiological agents associated with fresh produce–related infection *(7)*. At least 12 well-documented outbreaks in the United States have involved tomatoes; tomatoes were responsible for 1,990 confirmed cases and were suspected in another 75,000 cases *(7, 19, 27)*. Outbreaks have involved diverse *Salmonella* serotypes, including Javiana, Montevideo, Baildon *(16),* Typhimurium *(6),* and Wandsworth *(7)*. In 2008, a multistate outbreak in the United States and Canada was caused by *Salmonella* Saintpaul. Jalapeño peppers were implicated in this outbreak, but serrano peppers and tomatoes also were believed to have contributed to it. The outbreak strain was detected in jalapeño peppers collected in Texas and also in water and serrano peppers on a Mexican farm in the state of Nuevo Leon. Tomato tracebacks did not converge on a source *(2)*.

Chili peppers are important commercial crops in Mexico; in 2009, more than 2 million tons were produced. Of this production, approximately 25 and 10% were jalapeño and serrano peppers, respectively *(26)*. These two

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pepper species are most commonly consumed raw in Mexico and other countries *(10)*. Tomato production in Mexico increased by 56.2% from 1994 to 2001, and during the winter, most of the tomatoes consumed in the United States come from Mexico *(18)*.

Produce imported from Mexico, although it has been shown to be of an equivalent microbial quality to domestic samples in the United States *(18)*, has been linked to several disease outbreaks in the United States *(5, 8, 9)*. Intestinal diseases due to consumption of contaminated food are among the top five causes of disease in Mexico *(9)*. Studies in central Mexico have found high levels of contamination on serrano and jalapeño peppers *(10, 25)*. Another study in northeastern Mexico conducted a brief analysis of different crops, including tomatoes and peppers, and found no pathogens *(14)*. However, that study did not analyze different varieties of tomatoes. The current study investigates the microbiological quality of two varieties of tomatoes (saladette and bola) and two varieties of peppers (jalapeño and serrano) in several retail establishments in the metropolitan area of Monterrey, in the state of Nuevo Leon, Mexico, during 2010 to 2011. Of particular interest was the analysis of pepper stems, which have been reported to have high levels of contamination *(21)*.

MATERIALS AND METHODS

Vegetable samples. The microbial contamination level of 160 produce samples of four products (serrano peppers, jalapeño peppers, bola tomatoes, and saladette [Roma] tomatoes) was assessed. Forty samples of each item were obtained (20 from local markets and 20 from supermarkets) from October 2010 to February 2011. Samples were collected in the metropolitan area of Monterrey, Mexico (including the cities of Monterrey, Guadalupe, San Nicolás, Escobedo, and San Pedro), placed in sterile bags, and transported on ice within 1 h to the laboratory. Samples were analyzed immediately upon arrival.

Sample preparation and microbial determination. Upon arrival at the laboratory, stems were separated from the peppers, and samples were analyzed separately. Samples (25 g) from each produce item were randomly removed and homogenized with 225 ml of buffered peptone water and then were pummeled for 2 min in a stomacher lab blender (Led Techno, Eksel, Belgium); serial dilutions were made from the blended material. For the pepper stems, 10 g of sample was homogenized with 90 ml of buffered peptone water, and the same protocol was followed.

To determine the level of aerobic mesophilic microorganisms, the Mexican official standard protocol (NOM-092-SSA1-1994 *(22)*) was used. Briefly, 1 ml of each serially diluted sample was placed into a petri dish. Then 20 ml of plate count agar (Difco, BD, Sparks, MD) heated to 45°C was added, and the mixture was gently homogenized. After solidification, plates were incubated (35 ± 1°C for 48 h), and colonies were counted.

For coliform and *E. coli* determination, the method described in the *Bacteriological Analytical Manual (12)* was followed. The serially diluted sample (1 ml) was placed in the center of a petri dish, 20 ml of violet red bile agar (Difco, BD, heated at 45°C) was added, and the mixture was gently homogenized. After solidification, the agar was overlaid with 5 ml of double-strength violet red bile agar supplemented with 4-methylumbelliferyl-β-D-glucuronide and was allowed to solidify. Inverted plates were incubated at 35°C for 24 ± 2 h, and red-purple colonies were counted and considered as total coliforms, whereas fluorescent colonies under UV light were considered to be *E. coli*. To determine fecal coliforms, red-purple colonies were inoculated in tubes containing 5 ml of *E. coli* broth (BD Diagnostic Systems, Franklin Lakes, NJ) with a Durham tube. After 48 h at 45°C, tubes showing growth and gas production were considered as positive.

To determine the presence of *Salmonella,* the Mexican official standard protocol (NOM-114-SSA1-1994 *(23)*) was used. For preenrichment, samples (25 g) were transferred into a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI), and 225 ml of sterile lactose broth was added. For pepper stem analysis, 10 g of stems and 90 ml of lactose broth were used. After mixing well by swirling, the pH was adjusted to 6.8 ± 0.2, followed by incubation for 24 ± 2 h at 35°C. Enrichment was made by transferring 1 ml of the mixture into a tube with 10 ml of Rappaport-Vassiliadis medium and 10 ml of selenite-cystine broth (both from Difco, BD). Incubation was carried out at 35 ± 0.2°C for 24 h. Next, a loopful of culture was streaked onto plates of xylose lysine desoxycholate agar, Salmonella Shigella agar, and brilliant green agar (all from Difco, BD). Plates were incubated for 24 ± 2 h at 35°C. Colonies suggestive of *Salmonella* on xylose lysine desoxycholate agar (pink colonies with or without black centers), brilliant green agar (pink to fuchsia colonies surrounded by red medium), or Salmonella Shigella agar (transparent to opaque colonies with or without black centers) were harvested and subjected to additional tests (lactose, glucose, and sucrose fermentation, lysine decarboxylation, sulfhydric acid, and urease production) and screened for the presence of the *invA* gene by PCR *(28)*). *Salmonella* Typhi ATCC 19430 and *Salmonella* Typhimurium ATCC 14028 were used as positive controls.

Statistical analysis. To determine whether the samples were normally distributed, the Kolmogorov-Smirnov test was used *(30)*. For mesophilic and total coliform microorganisms, one-way analysis of variance *(30)* was conducted to determine differences in levels among produce types, location, and kind of market. For fecal coliforms, the Kruskal-Wallis test *(30)* was followed, and for *Salmonella* spp., the Cochran test *(30)* was used. Differences were considered to be significant if $P \leq 0.05$. Analyses were performed using SPSS Statistics software (version 17.0, SPSS, Inc., Chicago, IL) and STATISTICA (version 7.0, Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

Detection of pathogenic microorganisms on contaminated produce is difficult for a variety of reasons, including the long time required, complicated methodologies, and high cost. The detection of representative indicator microorganisms is easier and may be used to signal the presence of pathogenic bacteria *(21)*. Our results showed that total viable counts (aerobic mesophilic microorganisms) for crops ranged from fewer than 10 to greater than 10^5 CFU/g (Figs. 1 to 3). Levels were higher in peppers (means of 4.4 and 4.7 log CFU/g for jalapeño and serrano, respectively) than in tomatoes; both varieties of tomato showed low levels of bacteria (means of 3.2 and 3.6 log CFU/g for bola and saladette, respectively) (Fig. 4). Differences ($P \leq 0.05$) in microbial load among produce varieties of both vegetables were observed; serrano peppers and saladette tomatoes had the highest levels. However, no differences were found between the market types (data not shown). The results obtained are in accordance with those reported by Gómez et al. *(14)* for

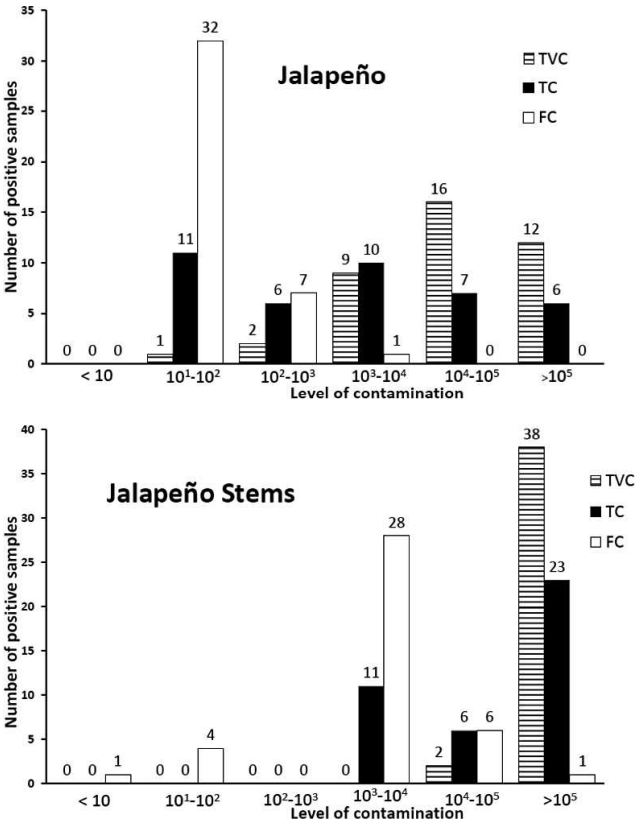

FIGURE 1. *Total viable counts of microorganisms (TVC), total coliforms (TC), and fecal coliforms (FC) present in jalapeño peppers and jalapeño stems, purchased in the metropolitan area of Monterrey, Mexico. Upper number indicates the number of positive samples.*

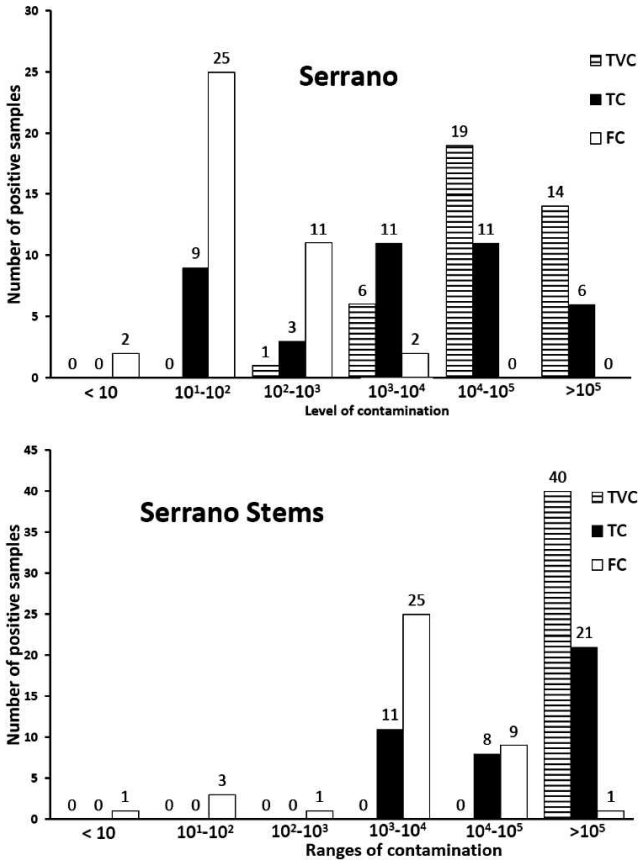

FIGURE 2. *Total viable counts of microorganisms (TVC), total coliforms (TC), and fecal coliforms (FC) present in serrano peppers and serrano stems, purchased in the metropolitan area of Monterrey, Mexico. Upper number indicates the number of positive samples.*

vegetables in northeastern Mexico; they reported counts lower than 10^4 CFU/g on tomatoes and reported differences in levels of mesophilic aerobic bacteria among different vegetables. Similarly, Liao et al. *(21)* reported that peppers obtained from grocers in Pennsylvania had levels of aerobic microorganisms ranging from 4.7 to 6.3 log CFU/g of tissue, with an average of 5.6; these levels are similar to those found in the present study. Another study conducted in the central part of Mexico (Pachuca, Hidalgo) reported higher levels of mesophilic aerobic bacteria in peppers at retail with counts of 7.2 and 6.4 log CFU/g for serrano and jalapeño peppers, respectively *(10)*. This level of contamination could be due to deficiencies in quality control systems of pepper production and distribution in that region.

The total coliform levels found in this study for all crops ranged from less than 10 to greater than 10^5 CFU/g (Figs. 1 to 3), with median values of 3.3, 3.7, 2.6, and 2.6 log CFU/g for jalapeño, serrano, bola, and saladette varieties, respectively (Fig. 5). Peppers (jalapeño and serrano) exhibited greater concentrations of coliform bacteria than tomatoes (Fig. 5); however, no differences ($P \leq 0.05$) were observed among varieties of each crop or the type of store where the samples were purchased. The levels of contamination found in this work were much less than those reported for jalapeño (3.0 to 8.1 log CFU per sample) and serrano peppers (log 4.5 to 8.5 CFU per sample) from popular markets in Pachuca, Hidalgo, Mexico *(10)*. On the other hand, the levels of fecal coliforms ranged from less than 10 to 10^3 log CFU/g for peppers and less than 10 to 10^2 log CFU/g for tomatoes (Figs. 1 to 3). In addition, the levels of fecal coliforms in this study were slightly less than those reported for jalapeño and serrano peppers in the central part of Mexico *(11)*. These differences in contamination could be due to variations in agricultural and handling practices as well as analysis methods.

Pepper stems accounted for a greater contamination level of aerobic mesophilic and total coliforms, when compared to the rest of the fruit (Figs. 1 and 2). These results are in accordance with those reported by Liao et al. *(21),* who determined the relative distribution of native microflora on two different parts of jalapeño peppers (calyx [stem] and flesh pod) and demonstrated that most of the contamination sites were located at the calix (stem) (>80%), while only a small proportion (<10%) was recovered from the flesh pod. The wrinkled texture of the stem may better suit it to harbor microorganisms than the smooth texture of the comestible area. Furthermore, the stem is the contact area of pepper used to pluck it from the plant during harvest.

Mexico is divided into three major regions for pepper production: the north-northeast region with high levels of technology, the central (Bajío) region with medium levels of

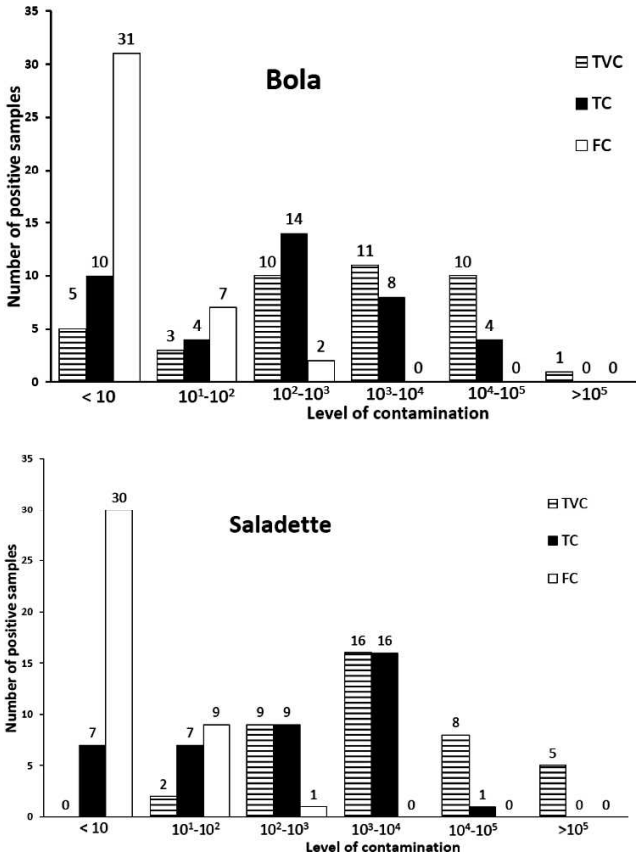

FIGURE 3. *Total viable counts of microorganisms (TVC), total coliforms (TC), and fecal coliforms (FC) present in bola and saladette tomatoes purchased in the metropolitan area of Monterrey, Mexico. Upper number indicates the number of positive samples.*

technology, and the south-southeast region with low levels of technology *(1)*. These conditions could also help to explain the differences in contamination of peppers found in central and northern Mexico.

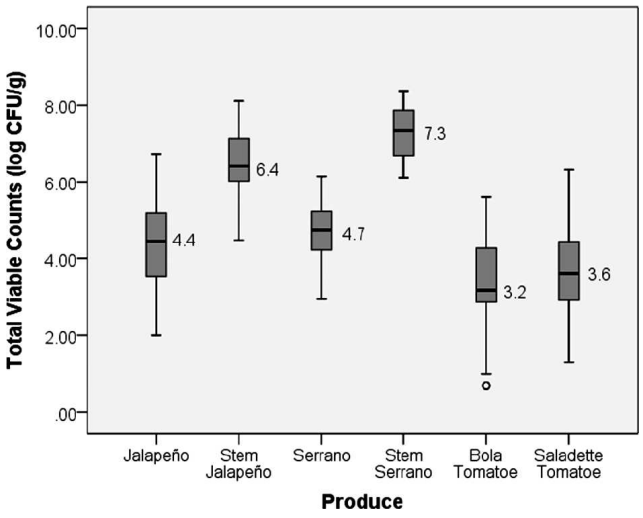

FIGURE 4. *Total viable counts of microorganisms present in fresh produce purchased in the metropolitan area of Monterrey, Mexico. The box represents 75% of the samples. The line inside each box indicates the geometric mean.*

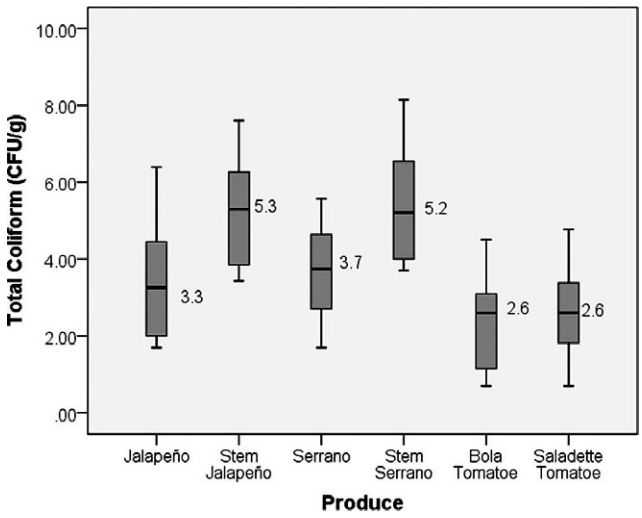

FIGURE 5. *Total coliform counts present in fresh produce purchased in the metropolitan area of Monterrey, Mexico. The box represents 75% of the samples. The line inside each box indicates the geometric mean.*

Historically, outbreaks of salmonellosis in humans have been attributed to consumption of contaminated tomatoes, mustard cress, bean sprouts, cantaloupe, and watermelon. However, in the summer of 2008, consumption of *Salmonella*-contaminated jalapeño peppers was implicated in one of the largest reported foodborne illness outbreaks *(8)*. Analysis of the presence of pathogenic bacteria in our samples showed that only one sample of bola tomatoes (1.25%) and one of jalapeños (1.25%) were positive for *Salmonella* (confirmed by PCR). However, the pepper contamination was found in the stem, which usually is removed before consumption. The frequency of positive samples for *Salmonella* in tomatoes and peppers is usually low *(19),* which is in accordance with our results. In conclusion, the results of our study show that the level of contamination of peppers and tomatoes is low in the metropolitan area of Monterrey, Mexico.

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