

PEER-REVIEWED ARTICLE

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***Salmonella* and *E. coli* O157:H7 Prevalence and Generic *E. coli* and Coliform Quantitative Baseline in Raw Pork and Beef in Retail Channels in Mexico**

ABSTRACT

This project was designed to determine the prevalence of *Salmonella* and *E. coli* O157:H7 and to quantify generic *E. coli* and coliforms in fresh whole beef, whole pork, ground beef and ground pork from various retail channels (supermarkets, city markets, street vendors, and butcher shops) in three major cities in Mexico (Mexico City, Guadalajara and Monterrey). The overall prevalence of *Salmonella* was found to be 4.4, 7.8, 7.3, and 13.9% in whole beef, whole pork, ground beef, and ground pork, respectively. With regard to retail channels, supermarkets had the lowest *Salmonella* prevalence in the samples collected (1.3%), followed by butcher shops (8.4%), whereas street vendors and city markets had the highest (13.6 and 22.3%, respectively). Analysis by city indicated that Monterrey had the lowest prevalence, followed by Mexico City, while Guadalajara showed the highest prevalence. *E. coli* O157:H7 was not detected in any of the samples; however, coliforms ranged

from 0.60 to 7.30 log CFU/g. This baseline for the prevalence of *Salmonella* and *E. coli* O157:H7 in various cities and retail venues will help in the establishment of controls during the processing of these types of products and serve as a starting point to create trend analyses, performance criteria, and microbial risk assessments in Mexico.

INTRODUCTION

Foodborne disease is the principal cause of mortality of pre-school children (1 to 4 years) in Mexico (6, 12, 16). In children ages 5 to 14, foodborne illness is the 10th leading cause of death (6). Between 1993 and 2002, 63 foodborne outbreaks were reported in Mexico, affecting 12,748 people (16). The causative agents were reported to be 41.35% viral, 35.86% bacterial, 14.77% plant tissue poisoning, 5.91% chemical, and 1.27% seafood toxin (16). The bacterial agents reported most frequently were *Salmonella* (15.9%), *Escherichia coli* (4.64%), and *Staphylococcus* (6.33%) (12). However, these numbers were most likely underestimated as a result of inadequate surveillance systems and under-reporting (16).

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Coordination between industry and government officials to establish accurate reporting systems will allow officials to obtain accurate epidemiological data that can be used for decision making and development of policies that could reduce the high occurrence of foodborne illnesses in Mexico.

Controls in the food chain can directly affect the transmission of foodborne pathogens; however, a lack of sanitary regulations in any segment of the industry can lead to foodborne illness outbreaks. Currently, food safety regulations in Mexico are monitored by the Mexican government and implemented in some processing facilities (1). These regulations are called Tipo Inspección Federal (TIF) (16). Processing facilities under TIF regulation, often referred to as “TIF processing facilities,” are similar to the U.S. processing facilities that are monitored by the United States Department of Agriculture, Food Safety Inspection Service (USDA-FSIS) in that they have similar food safety guidelines and regulations (8). Processing facilities certified by TIF are eligible to export meat products to other countries and supply products to supermarkets, hotels, and select high-end restaurants in Mexico. For processing facilities not following TIF regulation, food safety regulations are not implemented. Many of these facilities are privately owned and municipal processing facilities and are referred to as non-TIF processing facilities. At these locations, there is oversight of animal health but not of food safety conditions. Non-TIF regulated processing facilities supply meat products to retail channels such as city markets, butcher shops, and street vendors throughout Mexico but are not allowed to supply supermarkets or restaurants (2). Many non-TIF processing facilities may lack the minimum requirements to guarantee sanitary operating conditions and may have poor hygienic conditions during meat processing, resulting in an unsafe food supply that can directly affect public health in Mexico (11). Determining the sources of pathogens in the food supply will help identify areas of concern related to implementation of control measures to reduce contamination and foodborne illnesses in Mexico.

The objective of this study was to determine the prevalence of *Salmonella* and *E. coli* O157:H7, along with the generic *E. coli* and coliform quantitative baselines, in beef and pork products collected in various retail channels in three major cities in Mexico.

MATERIALS AND METHODS

Collection of samples

The prevalence of *Salmonella* and *E. coli* O157:H7 and the enumeration of generic *E. coli* were determined in Mexican fresh beef and pork—ground and whole muscle products—at four different types of retail channels (supermarkets, popular city markets, street vendors and butcher shops) in the most populous cities of Mexico: Mexico City, Guadalajara, and Monterrey. To guarantee the distribution of representative samples, each of the available retail channels was sampled

three times in each of four geographical zones (north, south, east, and west) within each city. Samples purchased from each retail channel consisted of whole muscle beef (WB) (inside round), whole pork (WP) (loin), ground beef (GB), and ground pork (GP), if available.

Meat samples were transported cold in disposable insulated coolers containing frozen gel-packs. The samples were transported to laboratories at local universities for consolidation, swabbing, and packaging. Whole meat samples were swabbed using a template that allowed collection of an area of 100 cm². A cellulose biocide-free sponge containing 10 ml of Buffered Peptone Water (BPW) was used; the sponge was attached to a plastic handle and sealed in a 24 oz sterile plastic bag. Ground beef and pork were transferred to sterile bags and labeled. All samples (swabs and ground beef and pork) were stored in refrigeration at ca. 4°C and then transported by air in insulated sealed coolers containing frozen gel-packs that maintained the low temperature until the samples arrived at Texas Tech laboratories for further analysis.

Detection of *Salmonella* and *E. coli* O157:H7

The detection of both *Salmonella* and *E. coli* O157:H7 was conducted using the real time polymerase chain reaction (PCR) BAX® System (DuPont Qualicon, Wilmington, DE), an automated standard PCR-based method that is highly sensitive; it is AOAC approved and has been adopted by the USDA-FSIS as a standard method for detection of *Salmonella* and *E. coli* O157:H7 in swabs collected from fresh beef and pork samples. The detection limit after enrichment is 10⁶ cells per sponge (5). For both *Salmonella* and *E. coli* O157:H7, swab samples were pre-enriched in Tryptic Soy Broth (TSB—EMD Millipore Chemicals; Darmstadt, Germany) at 37°C for 18–24 h. All samples were subjected to standard BAX protocols as described in the BAX guidebook, for the presence of either *E. coli* O157:H7 or *Salmonella* (14).

Enumeration of samples

Twenty percent of the samples were subjected to enumeration of total generic *E. coli* and coliforms. To perform the enumeration, sponge samples, 10 g ground beef, and 10 g ground pork were diluted serially in Buffered Peptone Water (BPW—EMD Millipore Chemicals; Darmstadt, Germany), and plated on Petrifilm (3M, St. Paul, MN) for coliforms and generic *E. coli*. Plates were incubated at 37°C for 24 to 48 h and total typical colonies were counted. Estimated populations were recorded as colony-forming units, transformed to log, and recorded.

Statistical analysis

A Chi-square analysis was conducted on the overall prevalence of *Salmonella* for each city by a commercially-available statistical analysis program (SAS Institute Inc., Cary NC). Frequency tables were created to identify the percentage of positives in order to report *Salmonella* prevalence.

RESULTS

The total number of samples collected for this project was 1,154. The availability of meat in the retail channels was the limiting factor in obtaining the same number of samples from each retail channel. In each city, 100 to 150 samples were collected each day over a 2 to 3 day period. At least 318 samples were collected in each city, which was associated with the cuts available and the types of retail channels accessible for sampling. Samples were collected in two separate visits to each city.

Mexico City had all four retail channels—butcher shops, street vendors, city markets, and supermarkets (Fig. 1). The butcher shop samples consisted of 33 whole beef, 26 whole pork, 21 ground beef and 16 ground pork; street vendor samples 31 whole beef, 24 whole pork, 19 ground beef and 14 ground pork; city market samples 28 whole beef, 20 whole pork, 17 ground beef and 19 ground pork and supermarket samples 68 whole beef, 40 whole pork, 30 ground beef, and 24 ground pork. Monterrey had only two of the retail channels—supermarkets and butcher shops (Fig. 2). Supermarket samples consisted of 88 whole beef, 70 whole pork, 42 ground beef, and 22 ground pork, whereas butcher shop samples consisted of 83 whole beef, 54 whole pork, 32 ground beef, and 15 ground pork. Guadalajara

had three retail channels—supermarkets, city markets, and butcher shops—but did not have street vendors (Fig. 3). Supermarket samples consisted of 72 whole beef, 58 whole pork, 38 ground beef, and 34 ground pork; city market samples 18 whole beef, 17 whole pork, 15 ground beef and 14 ground pork, and butcher shop samples 13 whole beef, 13 whole pork, 13 ground beef, and 13 ground pork.

Salmonella point prevalence

Overall, the prevalence of *Salmonella* in the samples analyzed in this study was 4.4% in whole beef, 7.8% in ground beef, 7.3% in whole pork, and 13.9% in ground pork. Figure 1 represents the distribution of the prevalence by city and type of sample collected (WB, WP, GB, and GP). Guadalajara appears to be the city with the highest *Salmonella* prevalence in all retail channels, while Monterrey had the lowest, during this study. With respect to the retail channel, city markets had the highest prevalence (22.3%), whereas supermarkets had the lowest (1.6%), during this study (Fig. 2).

Analysis by city indicated that in Mexico City (Fig. 3, Table 1), the supermarkets had the fewest *Salmonella*-positive samples for all products compared with the other retail channels (Table 1). The prevalence of *Salmonella* in supermarkets was

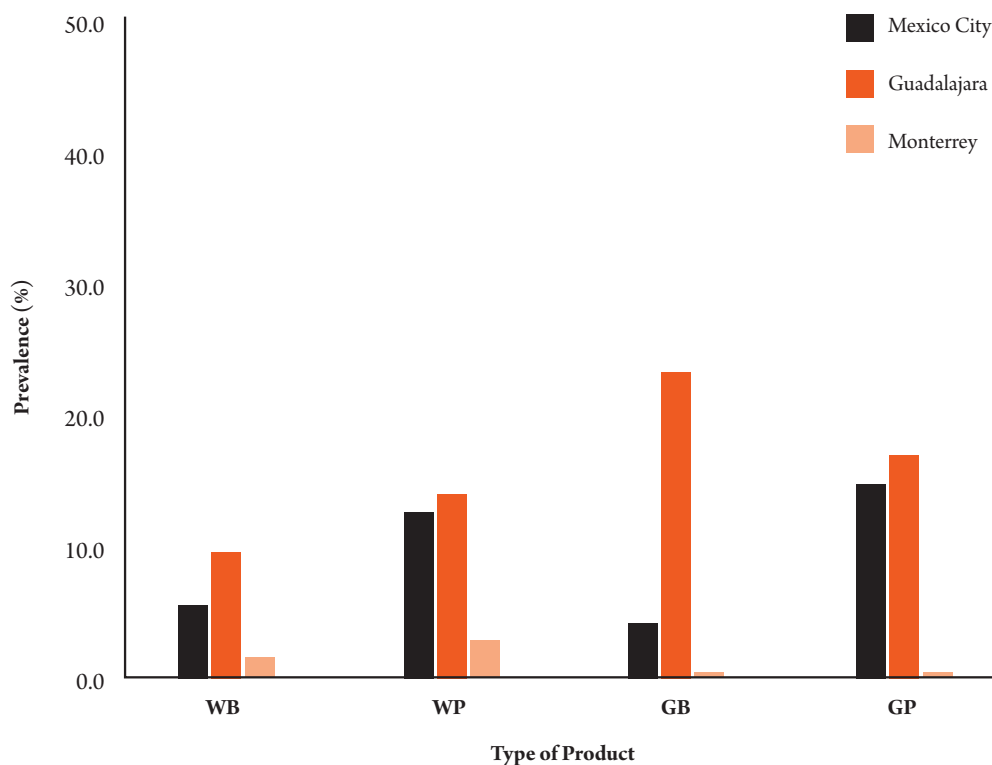


Figure 1. Overall prevalence of *Salmonella* found in each city from different types of samples analyzed: WB, whole beef; WP, whole pork; GGB, ground beef; GP, ground pork.

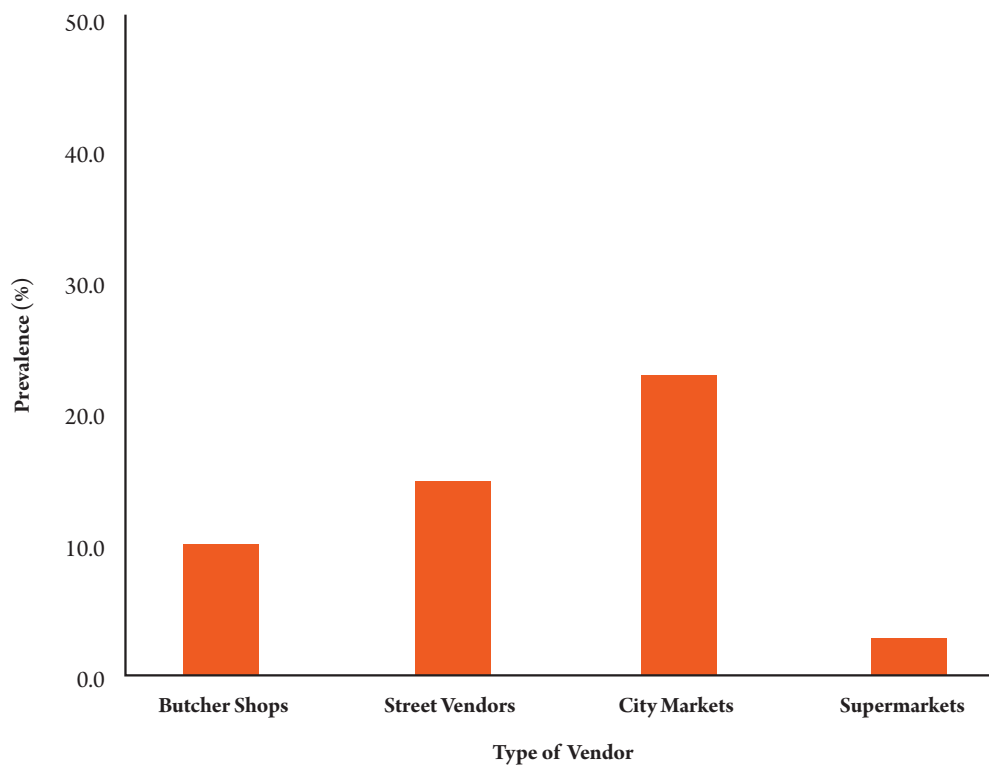


Figure 2. Overall prevalence of Salmonella by retail channel. Data represent percentages of samples identified positive for Salmonella from the total samples obtained.

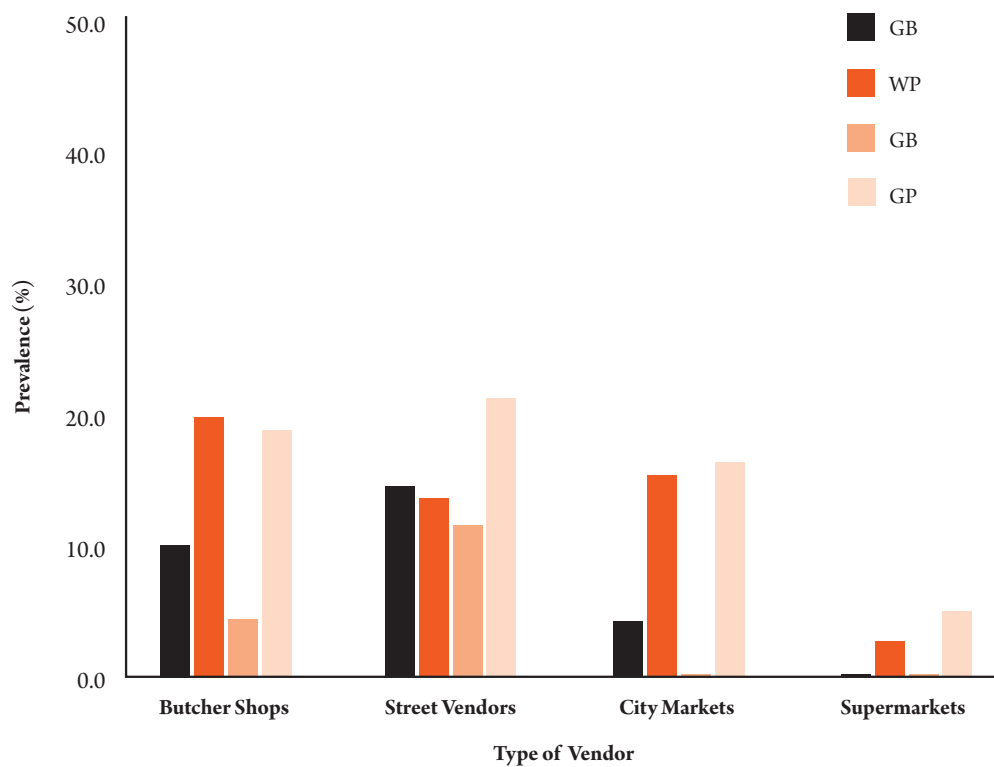


Figure 3. Overall prevalence of Salmonella from samples obtained at different types of markets in Mexico City. Type of product: WB, whole beef; WP, whole pork; GGB, ground beef; GP, ground pork.

TABLE 1. *Salmonella* prevalence at different retail channels in Mexico City

	Butcher shops		Street vendors		City markets		Supermarkets	
	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)
WB	33	9.1	31	12.9	28	3.6	68	0.0
WP	26	19.2	24	12.5	20	15.0	40	2.5
GB	21	4.8	19	10.5	17	0.0	36	0.0
GP	16	18.8	14	21.4	19	15.8	24	4.2

WB, whole beef; WP, whole pork; GB, ground beef; GP, ground pork

^aTotal of samples collected per city, per retail channel

^bPercentage of *Salmonella* per retail channel

0.0% (0/68) in whole beef, 0.0% (0/30) in ground beef, 2.5% (1/40) in whole pork, and 4.2% (1/24) in ground pork. *Salmonella* prevalence in, ground beef was lowest, with 0% positive (0/30), in supermarkets, 0.0% positive (0/17) in city markets, 4.8% positive (1/21) in butcher shops, and 10.5% positive (2/19) in meat sold by street vendors ($P < 0.01$). The prevalence of *Salmonella* in whole beef was 9.1% positive (3/33) in butcher shops, 12.9% positive (4/31) in meat from street vendors and 3.6% positive (1/28) in meat from city markets. The prevalence of *Salmonella* in whole pork was 19.23% positive (5/26) for butcher shops, 12.5% positive (3/24) for street vendors and 15% positive (3/20) for city markets. The prevalence of *Salmonella* in ground pork products was the highest in all retail channels except butcher shops. In Monterrey (Fig. 4, Table 2), no *Salmonella* was detected in whole pork, ground beef, or ground pork purchased from butcher shops. The prevalence of *Salmonella* in whole beef collected in Monterrey was 1.2% (1/83) in butcher shops. In supermarkets, no *Salmonella* was detected in ground beef or ground pork but was detected at 1.1% (1/88) in whole beef and 2.9% (2/70) in whole pork. In Monterrey, samples were not obtained from street vendors nor from city markets, as the city does not allow meat to be sold in this type of establishment. In Guadalajara (Fig. 5, Table 3), the prevalence of *Salmonella* in all products purchased from supermarkets was lowest compared with each of the other retail channels, ranging from 0% positive (0/38) in ground pork to 5.26% positive (2/38) in ground beef ($P < 0.01$). *Salmonella* prevalence in butcher shops was 46.15% positive (6/13) in ground beef, 23.08% positive (3/13) in ground pork, 15.38% positive (2/13) in whole beef and 23.08% positive (3/13) in whole pork. City markets had the highest percentage of *Salmonella* positive samples, ranging from 33.33% positive

(6/18) in whole beef to 50.0% (7/14) in ground pork ($P < 0.01$). Overall, for all the cities sampled in Mexico, supermarkets had the lowest percentage of samples testing positive for *Salmonella*, with the exception of Monterrey ($P < 0.01$). In Monterrey, *Salmonella* was detected in 2.86% (2/70) of whole pork samples tested.

***E. coli* O157:H7 point prevalence**

No *E. coli* O157:H7 was found in any samples collected in any city of Mexico.

Total coliforms

A total of 254 samples, corresponding to 20% of the samples collected, were enumerated. In Mexico City, total coliform counts in whole beef samples ranged from 1.84 log CFU/cm² for supermarkets to 4.36 log CFU/cm² for city markets. The overall average from all retail channels was 3.82 log CFU/cm² ($n = 35$). Whole pork samples had the lowest bacterial concentration, 2.52 log CFU/cm², in supermarkets, and the highest, 5.13 log CFU/cm², city markets. The overall average for all retail channels was 4.54 log CFU/cm² ($n = 22$). Coliform counts in ground beef samples ranged from 2.61 log CFU/g in city markets to 3.38 log CFU/g in supermarkets, and the overall average for all retail channels was 3.13 log CFU/g ($n = 29$). Coliform counts in ground pork samples ranged from 2.60 log CFU/g in supermarkets to 3.43 log CFU/g in city markets, and the overall average for all retail channels was 3.19 log CFU/g ($n = 21$). Overall, supermarkets had the lowest averages for all meat products, with the exception of ground beef. In Monterrey, coliform counts in whole beef samples ranged from 3.49 log CFU/cm² in supermarkets to 5.18 log CFU/cm² in butcher shops, and the overall average from both retail channels was 4.89 log CFU/

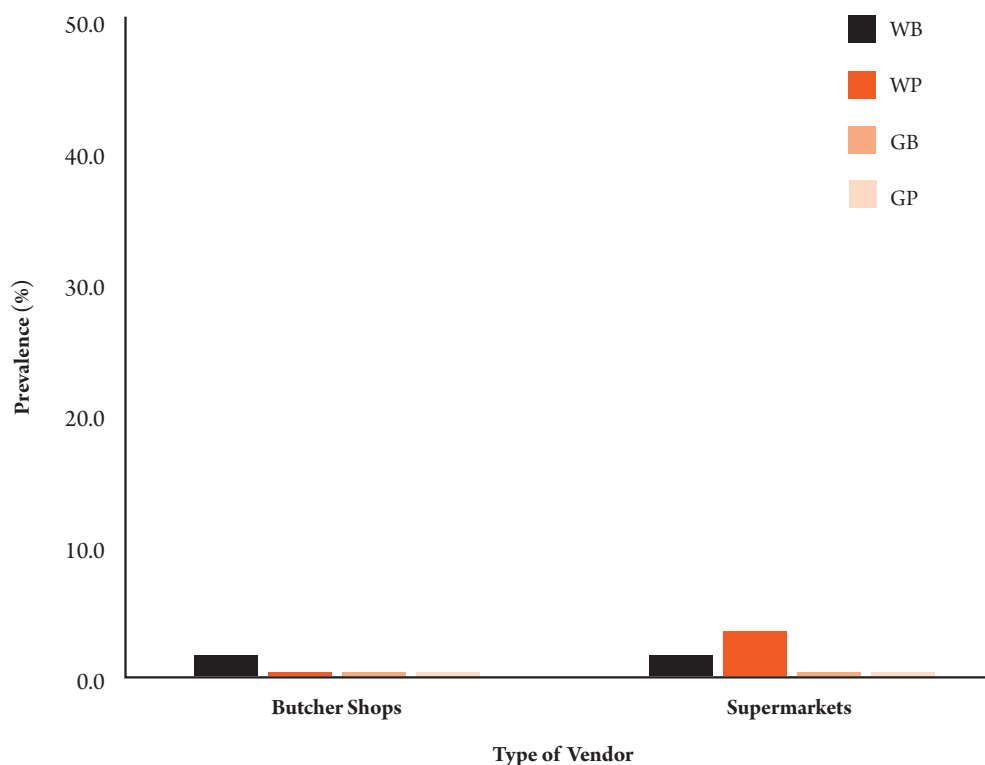


Figure 4. Overall prevalence of *Salmonella* from samples obtained at different types of markets in Monterrey. Type of product: WB, whole beef; WP, whole pork; GB, ground beef; GP, ground pork.

TABLE 2. *Salmonella* prevalence at different retail channels in Monterrey

	Butcher shops		Street vendors		City markets		Supermarkets	
	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)
WB	83	1.2	na		na		88	1.1
WP	54	0.0	na		na		70	2.9
GB	32	0.0	na		na		42	0.0
GP	3	0.0	na		na		7	0.0

WB, whole beef; WP, whole pork; GB, ground beef; GP, ground pork

^aTotal of samples collected per city, per retail channel

^bPercentage of *Salmonella* per retail channel

na, sample not available at that retail channel

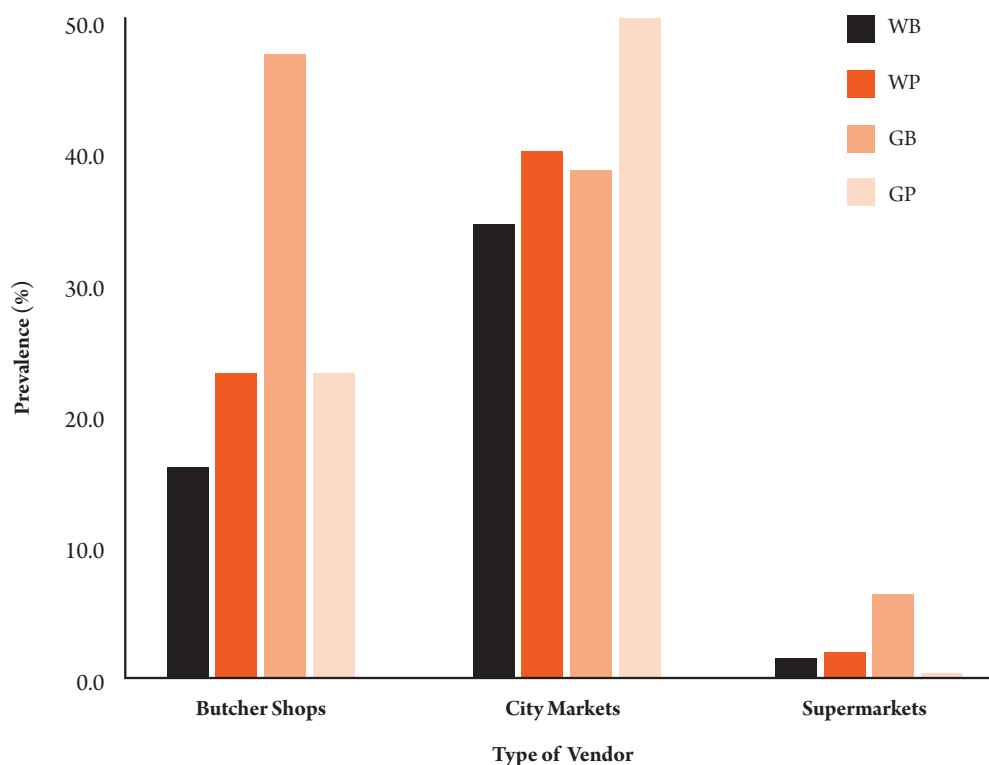


Figure S. Overall prevalence of Salmonella from samples obtained at different types of markets in Guadalajara. Type of product: WB, whole beef; WP, whole pork; GB, ground beef; GP, ground pork.

TABLE 3. Salmonella prevalence at different retail channels in Guadalajara

	Butcher shops		Street vendors		City markets		Supermarkets	
	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)	Samples ^a	Prevalence ^b (%)
WB	13	15.4	na		6	33.3	72	1.4
WP	13	23.1	na		7	41.2	58	1.7
GB	13	46.2	na		6	40.0	38	5.3
GP	13	23.1	na		7	50.0	34	0.0

WB, whole beef; WP, whole pork; GB, ground beef; GP, ground pork

^aTotal of samples collected per city, per retail channel

^bPercentage of *Salmonella* per retail channel

na, sample not available at that retail channel

cm² (n = 38). For whole pork samples, coliform counts were similar in samples collected from butcher shops and from supermarkets, 3.64 log CFU/cm² in (n = 37). The coliform counts in ground beef samples ranged from 5.89 log CFU/g in butcher shops to 6.42 log CFU/g in supermarkets, and the overall average for both retail channels was 5.01 log CFU/g (n = 33). Coliforms in ground pork samples ranged from 6.61 log CFU/g in supermarkets to 7.3 log CFU/g in butcher shops, and the overall average for both retail channels was 5.81 log CFU/g (n = 37). Overall, butcher shops had the lowest averages for ground beef and pork products, but higher averages were seen in whole beef samples. Samples were collected in Guadalajara, but coliforms were not enumerated as a result of shipping delay (more than 5 days), resulting in loss of cold storage conditions. The samples were subjected to *Salmonella* and *E. coli* O157:H7 detection only.

Generic *E. coli* enumeration

Samples enumerated for coliforms, corresponding to 20% of the total collected, were also enumerated for *E. coli*. In Mexico City, the generic *E. coli* in whole beef samples ranged from 0.15 log CFU/cm² in supermarkets to 2.83 log CFU/cm² in butcher shops, and the overall average from all retail channels was 2.42 log CFU/cm² (n = 46). *E. coli* in whole pork samples ranged from 1.16 log CFU/cm² in supermarkets to 4.8 log CFU/cm² in city markets, with an overall average for all retail channels of 4.21 log CFU/cm² (n = 22). Ground beef samples contained generic *E. coli* ranging from 1.9 log CFU/g in supermarkets to 2.56 log CFU/g in street vendors, with an overall average for all retail channels of 2.35 log CFU/g (n = 28). Generic *E. coli* counts in ground pork samples ranged from 0.99 log CFU/g in supermarkets to 2.75 log CFU/g in street vendors, and the overall average for all retail channels was 2.45 log CFU/g (n = 21). Overall, supermarkets had the lowest averages for all meat products. In Monterrey, the generic *E. coli* in whole beef samples ranged from 0 log CFU/cm² in supermarkets to 2.64 log CFU/cm² in butcher shops, and the overall average for both retail channels was 2.34 log CFU/cm² (n = 39). Generic *E. coli* in whole pork samples ranged from 2.65 log CFU/cm² in butcher shops to 2.97 log CFU/cm² in supermarkets, and the overall average for both retail channels was 2.84 log CFU/cm² (n = 29). In ground beef samples, values ranged from 3.21 log CFU/g in supermarkets to 4.38 log CFU/g in butcher shops, with an overall average for both retail channels of 4.10 log CFU/g (n = 42). Ground pork samples *E. coli* counts ranged from 4.47 log CFU/g in supermarkets to 4.92 log CFU/g in butcher shops, with an overall average for both retail channels of 4.75 log CFU/g (n = 33). Overall, supermarkets had the lowest averages for all meat products with the exception of whole pork.

DISCUSSION

According to the World Health Organization (19), *Salmonella* is responsible for causing tens of millions of cases of human illness worldwide every year, and in the U.S. alone, causes 1.2 illnesses and hundreds of deaths (4). Unfortunately, in many developing countries, surveillance data on food-transmitted illnesses is not reliably available. Foodborne and zoonotic diseases are under-reported and considered a low priority (3), which makes it difficult to identify a point of reference with regard to foodborne pathogens prevalence. Studies conducted in Mexico suggest that *Salmonella* represents a public health issue that needs to be addressed (13, 19). In 2007, Paniagua et al. analyzed samples collected from 300 Mexican children diagnosed with diarrhea, ages 2 to 12 years old. A multiplex PCR was used for the detection of *E. coli*, *Salmonella*, and *Shigella* spp. Additionally, conventional methods for detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Giardia intestinalis* were used. All diarrheal samples were positive for one or two enteropathogens. In 2009, Estrada-Garcia et al. conducted a study in Mexico City to determine asymptomatic infection and acute diarrhea associated with diarrheagenic *Escherichia coli* pathotypes (DEP) in 76 children less than two years old. Through the use of a pathogen-specific multiplex PCR, 125 of the 795 stool samples tested positive for DEP (16% positive). The authors concluded that diarrheagenic *Escherichia coli* were major causes of diarrhea in Mexican children (6). The most common enteropathogens associated with illness in children included *E. histolytica* and *E. dispar*, at 70.3% positive; *Salmonella* Ohio, at 28.3% positive; *S. Typhimurium*, at 16.3% positive; *S. Infantis*, at 8% positive, *S. Anatum*, at 0.6% positive; *S. Newport*, at 0.3% positive; *G. intestinalis*, at 33% positive; *Escherichia coli* ETEC, at 13.3% positive; *Escherichia coli* EPEC, at 9.3% positive; *Escherichia coli* VTEC, at 8.5% positive, *Escherichia coli* EIEC, at 1% positive, *S. flexneri*, at 1.6% positive; and *S. sonnei*, at 1% positive (12). These data suggest that a variety of foodborne pathogens cause illnesses in Mexico. Even with limited surveillance, it is apparent that both *E. coli* and *Salmonella* are responsible for outbreaks in Mexico. However, very little information has been published on the prevalence of *Salmonella* in meats in Mexico. Therefore, the development of a pathogen baseline in meat products within Mexico can lead to implementation of control measures for the pathogens.

The data presented in this baseline study indicate that retail channels, other than supermarkets, could contribute significantly to foodborne illness in Mexico. It is apparent that retail channels selling beef and pork products supplied from municipal and individually-owned processing facilities have a higher prevalence of *Salmonella*, possibly due to variations in regulation. In Mexico, supermarkets are under TIF guidelines, whereas butcher shops, street vendors, and city markets are not. TIF establishments handle 51%

of the meat and poultry processed in Mexico and are considered to have higher sanitary standards than other types of establishments. In Mexico City there are about 50 TIF establishments; in Monterrey 20, and in Guadalajara 8; however, only a very small portion are certified to export to the United States (15, 18). These establishments, regulated by SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria), the National Service of Health, Food Safety, and Food Quality, are required to implement good manufacturing practices, to follow specific plant design requirements, and to base their food safety systems on HACCP (Hazard Analysis Critical Control Points) principles (18). Therefore, it is expected that the application of such regulations and the inspections conducted by the regulatory agency, would guarantee the safety of beef and pork products.

Hartzog-Hawkins (7), assessed non-TIF facilities in Mexico to identify food safety gaps and training needs; they also evaluated the link between *Salmonella* prevalence in these plants and food safety training and showed the lack of implementation of food safety regulation in non-TIF facilities. They also demonstrated that by providing training, food safety behavior improved and *Salmonella* prevalence was reduced (7). According to a report prepared by USDA (18), a surveillance study conducted in Mexico provided evidence that *Salmonella* spp. is a health risk of great magnitude in this country. During that study, in which samples were collected from 64 cities within different states of Mexico, a prevalence of *Salmonella* spp. of 36.4% and 29.9% was found in pork meat and beef, respectively. The study also reported that meat contamination was more common in states with greater levels of poverty (20). Miranda et al. (10) collected samples from supermarkets and retail stores in Mexico to evaluate the prevalence of *Salmonella*; their findings suggest

a prevalence of the pathogen in 17.3% of the pork samples and 15.1% of the beef samples (10). In another study, 2659 culture-confirmed *Salmonella* infections were identified in travelers who had visited other countries; of the 107 reported countries, Mexico was associated with 38% of the travel-associated infections (9). In addition, levels of coliforms and *E. coli* identified during this study revealed the necessity to improve hygienic practices during meat processing and commercialization, and should be used as an indicator of potential contamination with undesirable microorganisms.

Further research needs to be conducted to follow product back to its original source and processing facility. This approach would increase the opportunity to achieve less food contamination of food in Mexico. It would be beneficial to implement sanitary programs to improve food safety in retail channels to protect against foodborne illness. It would also be beneficial to implement interventions, such as refrigeration or chemical treatments, in the city markets to prevent pathogen growth or to reduce the pathogen prevalence on the products. Finally, it would be important to educate consumers about food safety and utilize educational programs in the retail channels to target the Mexican population. In the future, this baseline data will be used to identify points of control that can improve public health and food safety in Mexico.

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