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Article

Microbiological Risk Assessment of High-Demand Foodfrom Three Major Cities in Ecuador

Enrique Salazar Llorente, [1.*] *Fernando Cobos Mora* [1], *Luis Caicedo Hinojoza* [2], *Maribel Vera Suarez* [1].

1 *Faculty of Agricultural Sciences, Technical University of Babahoyo, Km 7.5 Vía Babahoyo – Montalvo.*

2 *Faculty of Health Sciences, Technical University of Babahoyo, Av.Uni-versitaria Km 1/2 via Montalvo*

Correspondence: ejsalazar@utb.edu.ec

ABSTRACT

The current study was carried out to estimate the risk of disease probability from the consumption of foods such as meats, poultry, unpasteurized cheeses, fruit-based drinks, ready-to-eat fruits, and typical preparations such as Encebollado, ceviche, and Bolón de Verde contaminated with *Salmonella,Escherichia coli* and *Listeria monocytogenes* in Ecuador using a quantitative microbiological risk assessment (QMRA).
A first-order Monte Carlo simulation probabilistic distribution approach was adopted to assess the occurrence of pathogens in the tested foods. [27]
The scenario was simulated using the concentration levels concerning the contaminant and food consumption obtained through an online survey with a sample size of 202 people.
A model (100,000 iterations) was run and created in an Excel spreadsheet using @Risk software. The results obtained are the risk of infection (possibilities of becoming infected by eating the food evaluated) and the contaminant dose per portion consumed.
Additionally, an exponential model with a single dose was used for risk characterization to determine the probability of becoming ill from contaminated food.
The QMRA model performed a prediction for the mean risk of Salmonella infection from ground beef consumption of 1.33 E - 04 log 10 cfu / serving, while the exponential model estimated a valueof 1.0 log cfu - serving. In the case of *Listeria monocytogenes, the* QMRA estimated an average probability of infection in unpasteurized fresh cheese of 5.9E-05 compared to the average disease risk estimated in the risk characterization for *L. monocytogenes* of 9.50E-13.
The QMRA estimated an average risk of infection by *E. coli* for Encebollado and ceviches of 5.6E -03 compared to the average risk of disease estimated in the risk characterization for *Escherichia coli* of 0.387 log cfu -ration. These results suggest the need to adopt effective mitigation strategies. Control parameters such as temperature during the supply chain and good hygiene practices during manufacturing can effectively control food-associated pathogens. More data is necessary toimprove the evaluation of the risk developed.

Keywords: Microbiological risk análisis, Infectious biological agents (Salmonella, *Listeria monocytogenes, Escherichia coli*), QMRA (Quantitative Microbial Risk Assessment), Typical foods in Ecuador, Population food consumption in three main cities of Ecuador.

INTRODUCTION

Foodborne pathogens are a global public health problem [1]. FoodNet 's preliminary report shows 25,606 infections, 5,893 hospitalizations, and 120 deaths from foodborne pathogens in 10 locations in the United States [2]. Many pathogens can potentially contaminate food, and there are many more scenarios in which such contamination could arise. Final product testing is time-consuming, expensive, often invasive and largely ineffective in ensuring the required level of food safety.

In this context, it should be noted that food safety is a significant problem with severe and lifelong implications for public health when food is consumed contaminated with pathogenic microorganisms [3]. During food preparation and safety considerations, Hazard Analysis Critical Control Point (HACCP) procedures monitor conditions and take necessary precautions to ensure food is safe to eat [4]. The predominant foodborne bacterial species that have been frequently associated with food include *Salmonella enterica, Escherichia coli, Campylobacter* species, Clostridium *species, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Shigella spp*, *Vibro parahaemolyticus* and *Yer- sinia spp,* [5]. *Listeria monocytogenes* represents a significant concern for ready-to-eat (RTE) foods, including raw milk cheeses, due to its high mortality rate, especially for those most at risk, e.g., pregnant women and immunocompromised individuals [6].

In 2017, EFSA reported 2,480 confirmed invasive human cases of listeriosis and a prevalence rate of 0.9% in soft and semi-soft cheeses made from raw or low-temperature-treated milk [7]. The presence of *Listeria monocytogenes* in raw milk and cheese has been widely reported, and its ubiquitous nature and contamination by distribution can occur at any stage of the production chain [8].

Salmonella spp still represents the EU's first causative agent of a foodborne outbreak: 9600 human cases and 2227 hospitalizations. Salmonella, the causative agent of foodborne outbreaks associated with the consumption of various types of cheese, can be detected in the intestinal tract of healthy animals, and contamination of milk occurs mainly during milking operations [9]

Escherichia coli is the major extended-spectrum beta-lactamase (ESBL)--producing bacterium identified in food products, humans, and environmental samples worldwide [10]. ESBL-producing E. coli strains in Ecuador have been characterized in clinical samples, raw vegetables, dog feces, and broiler chickens [11]. The growing demand for ready-to-eat (RTE) foods has significantly increased market demand for minimally processed foods, such as packaged salads, leading to large-scale production practices and a wide range of fresh products. The main driver of this market growth is the growing consumer demand for fresh, healthy and convenient products without additives [12].

In Ecuador, the consumption of ready-to-eat foods, as well as those that are prepared artisanally on the street, are very demanding in the main cities of our country by our population. However, these regulations do not restrict street vendors, who often operate without a legal permit. Street food vendors' poor knowledge and hygiene practices require significant improvements to ensure food safety.

In Guayaquil, Quito and Cuenca, Encebollado, Ceviches, Bolones, fruit salad, and fruit juice are usually eaten in the "hollows" where the hygienic and sanitary conditions are not met. Street-prepared products are more susceptible to microbial growth and cross-contamination because ingredients are less likely to be stored appropriately and relatively dirty utensils are used; lead time depends on your request. Mobile grocery vendors often have items at room temperature until a customer orders the same. Latency can be a critical factor in determining the likelihood of food poisoning after eating the product of your choice.

Based on the previously described, a quantitative microbiological risk assessment (QMRA) was carried out for 10 foods traditionally consumed in our country. The pathogens considered for this study were *Listeria* *monocytogenes, Escherichia coli and Salmonella* [13].

A quantitative microbial risk assessment (QMRA) is a science-based method that estimates the probability of acquiring a microbial infection by quantifying the concentrations of pathogenic bacteria present during each food preparation and consumption procedure [14].

Steps traditionally included in a QMRA are formulation of the problem, including deciding which microorganisms are of concern in which products (hazard identification), determination of intake hazards resulting from food consumption (evaluation of exposure) and the effect on population ex-posture (hazard characterization), and estimation of the overall risk for a specific population (risk characterization) [15].

This study estimated the risk of infection by *Listeria monocytogenes, Escherichia coli, and Salmonella due to the consumption of 10 types of high-demand foods in the cities of Guayaquil, Quito,* and Cuenca.

MATERIALS AND METHODS

Food consumption data

Data were collected on the consumption of foods traditionally consumed in our country, detailed inTable #1 concerning the level of risk and pathogen detected, such as ground beef, homemade sauces such as dressing, chicken, unpasteurized fresh cheese, Encebollado, ceviche, bolón, fruit salad, ready-to-eat fruits (grapes and strawberries) and fruit juices [16].

The sample size was 202 people from 18 to 25 years old (22.77% Guayaquil, 1.49% Quito, 3.47% Cuenca) 26 to 35 years old (17.8% Guayaquil, 1.49% Quito, 3.96% Cuenca) 36 to 45 years (28.7% Guayaquil, 8.42% Quito, 2.97% Cuenca) and 46 years and older (8.42% Guayaquil, 0.99% Quito). To record the data, an online survey was self-administered only once during October 2021 and was collected using Google Forms (Google, LLCMountain View, CA, USA). Participant responses are anonymous and confidential, following Google's privacy policy (*Https://Policies.Google.Com/Privacy?Hl=en*, n.d.).

The survey link was disseminated on social networks such as Facebook, Instagram and WhatsApp (Facebook, Inc. Merlo Park, CA, USA).

The online survey had 5 sections:

1. Age
2. City
3. The types of food consumed are based on the 10 proposed in this study.
4. Frequency of consumption
5. The size of portions of food consumed is based on those proposed.

The data needed to estimate exposure include the portion size and frequency of consumption of therelevant foods, combined with the frequency and concentration of pathogen contamination at the time of consumption.

To determine the size of food portions, the current recommendations are based on grams of food/food groups per day, so the analysis is based on food consumption in grams, as described by [17], the consumption range. The optimum for fruit is 200 to 400 g per day, fish and shellfish from 200 to 250 g daily, and red meat from 50 to 100 grams daily.

Food Contamination Data

Previously, ten groups of commonly consumed Ecuadorian foods were evaluated (Table 1); they were selected by taking 15 samples for each group from popular open-air markets in Quito, Guayaquil and Cuenca [16].

In summary, 450 samples (3 cities x 10 food groups x 15 samples) were taken and immediately sent in coolers with gel packs to the Centro de Investigaciones Biotecnológicas del Ecuador in Guayaquil for laboratory processing. After receipt, solid foods such as poultry, minced meat, perishable products, cheese, bolón, and fruits were aseptically cut into 2–5 g pieces. Broiler specimens were collected from fur-bearing areas, as suggested elsewhere [18]. For samples containing a mixture of liquidand solid foods such as ceviche, Encebollado, fruit salads, and sauces. 10 g of each sample containing parts of liquid and solid foods were aseptically weighed. We then pooled 10 g from three individual samples from the same food group and city to create a pooled sample.

Similarly, 10 ml of each juice sample was prepared for three separate samples from the same city. A total of 150 mixed samples were collected. Each mixed sample was transferred to a sterile, unfiltered bag containing 270 mL peptone water (Neogen, Lansing, MI) and homogenized for 1 min at 265 rpm in a Biomaster 80 stomacher (West Sussex, UK) to generate a sample. Broth suspension food fragments from stock suspensions were not used for serial dilution and plating.

To determine the presence of indicator populations of the microbiological quality of food such as total mesophilic aerobic populations, total coliforms, fecal coliforms, *Escherichia coli* (EC), *Salmonella spp* and *Listeria monocytogenes,* the protocols described in the local regulations for each type of microbiological analysis [19], and the methodology described by Salazar [16].

From the food matrices evaluated, the final value of Nmax (log cfu /g or ml) corresponding to the microbiological quality of each sample evaluated was generated (Table 2).

Food Consumption Exposure Assessment

A probabilistic approach was adopted following a first-order Monte Carlo simulation to assess food consumption exposure concerning *Escherichia coli, Salmonella and Listeria monocyte-genes.* In this study the serving size was 150 grams for ground beef, 30 grams for sauces, 160 grams of chicken, 40 grams of unpasteurized cheese, 430 grams of Encebollado, 430 grams of ceviche, 340 grams of bolón, 280 grams of fruit salad, 430 grams of ready-to-eat fruit (strawberry and grape), and 480 grams of fruit juices, this serving size for meat, fruit, and banana is in line with [16].

The daily consumption data and the concentration of microorganisms in the food portions were fitted to probability distributions. The best-fit distribution was selected based on the lowest Akaike Information Criterion (AIC) statistic and the probability-probability (PP) plot inspection. Dietary exposure distributions were modeled using a first-order Monte Carlo simulation based on 10,000 iterations.

Monte Carlo simulation is the most widely used tool in risk assessment, where a point estimate is replaced by a probabilistic distribution, reflecting reality with greater fidelity due to the more frequent selection of the most probable values. The more iterations are performed in the simulation, the greater the coverage of the statistical distribution field and, consequently, the more reliable the results will be.

The simulations were run three times to ensure adequate and reliable convergence. Distributions and Monte Carlo simulations were performed using the @Risk software package for Microsoft Excel version 7.5 (Palisade Corporation, USA). Results were reported as estimated daily exposure of each pathogen (E. *coli, Listeria monocytogenes, Salmonella)* expressed as log CFU /g/ day.

Table 3 shows input consumption data by type of food: Number of consumers (n), mean, minimum and maximum and distribution function of best-fit people.

City [to]	Food group [b]	*Escherichia coli*	*Salmonella*	*Listeria monocytogenes*
1	1	3.96	0.1	*
1	2	1.22	0.4	*
1	3	*	1	*
1	4	3.3	0.2	0.8
1	5	1.7	0.3	*
1	6	3.98	0.1	*
1	7	0.5	0.2	0.2
1	8	2.36	*	0.8
1	9	1.18	*	*
1	10	2,964	*	0.6
2	1	2.38	0.6	*
2	2	2.02	0.2	*
2	3	*	0.6	*
2	4	2.48	0.7	1
2	5	0.38	0.8	*
2	6	0.48	0.2	*
2	7	0.5	0.3	0.3
2	8	0.44	*	0.4
2	9	0.45	*	*
2	10	0.46	*	0.2
3	1	2.82	0.4	*
3	2	0.82	0.2	*
3	3	*	0.4	*
3	4	2.14	0.3	0.2
3	5	3.16	0.2	*
3	6	0.88	0.4	*
3	7	0.94	0.2	0.3
3	8	0.98	*	0.5
3	9	0.74	*	*
3	10	0.36	*	0.3

[a] Description of the city: 1 Guayaquil, 2 Quito, 3 Cuenca

[b] Food Group: 1 Ground beef, 2 Sauces, 3 Chicken, 4 Fresh cheese, 5 Encebollados, 6 Ceviche, 7 Bolón, 8 Fruit Salads, 9 Fruit, 10Fruit juice
*Not detected
Table 2. Average microbiological contamination (logUfc /g or ml)

Hazard characterization

To determine the characterization of the hazard, the dose-response relationship was established. Ingested doses or exposure risk was determined as the number of microorganisms (log cfu) in a serving of food at the time of consumption (grams). The dose-response model was performed to estimate the probability of infection from consuming a contaminated portion based on in-individual risk [20]. The data for this study were obtained from the interactions of the 10 types of food evaluated in the @Risk software simulation to the ingested dose, the value of which, based on probability, will be determined in the exposure assessment as the concentration of the consumption (log cfu in the portion consumed).

Risk characterization

For the characterization of the risk, a single-dose infection probability model was used for the dataobtained

from the simulation in the @risk software of the interaction between the concentration of the contaminant and the size of the portion of the ten types of food evaluated.
The dose-response model responds to the formula according to a study carried out [21 22].

Food type	Mean	Min	Max	Function
Ground beef	0.001664206	-0.002575954	0.006190638	RiskLaplace (0.0016667;0.00077012)
Sauces	0.000326801	0.000250002	0.00297064	RiskPareto (4.3435;0.00025)
Chicken	0.001847282	0.000190737	0.006467795	RiskExtvalue (0.00150268,0.00059788)
Fresh cheese	0.00049317	-4.96933E-06	0.001004871	RiskUniform (-0.00000497512;0.001005)
Encebollado	0.004330643	-0.00219275	0.01181695	RiskLaplace (0.0043333;0.0010105)
Ceviche	0.004352565	-0.004843119	0.01478736	RiskLaplace (0.0043333;0.0014819)
Bolón	0.001662605	-0.002093815	0.005780211	RiskLaplace (0.0016667;0.00060909)
Fruit salad	0.002503862	-0.007058545	0.01275962	RiskLaplace (0.0025,0.0014007)
Fruits	0.001871946	-6.12231E-05	0.0163066	RiskLoglogistic (-0.00035693;0.0020421;4.2406)
Fruit juice	0.003981067	-0.01237188	0.02230513	RiskLaplace (0.004;0.0023641)

Table 3: Input consumption data by type of food: number of consumers (n), average, minimumand maximum and distribution function of best fit, all expressed in g per serving

Single dose infection probability: 1 - exp (-r*D)
Where (r) is the probability of disease after consumption of a cell of *L. monocytogenes*, *Salmonellaspp,* and *Escherichia coli* and D is the consumed dose (number of cells per serving). The r-value for *Listeria monocytogenes* (2.37×10^{-14}) was taken as a reference from [23], *Salmonella enterica (0.59243*) calculated by [24], and for *Escherichia coli* (1.13×10^{-3}) from a study conductedby [21].
The ingested dose (D, CFU) was calculated by transforming the concentration from a logarithmic scaleto a natural number (10^) and multiplying by the serving size [25]. With this model, the probability of illness after consuming 5 servings of food during a week has been estimated in the present study.

RESULTS

Exposure assessment

The results obtained from the 202 people surveyed online in the cities of Guayaquil, Quito and Cuenca, 53% consume ground beef daily or weekly, 57% consume homemade sauces as dressings daily or weekly, 96.5% consume chicken daily or weekly, 58% consume unpasteurized fresh cheese daily or weekly, 57% consume Encebollado daily or weekly, 47% consume ceviche daily or weekly, 57.4% consume bolón daily or weekly, 67.3% of those surveyed consume fruit salad daily or weekly, 82.2% consume fruits such as grapes and strawberries daily or weekly and finally 82.7% consume fruit juices daily or weekly. Consumption levels for fruit salad, fruit, fruit juice, and meat or poultry consumption are similar to those reported in the Cape Town metropolitan area, South Africa, in 2013[26]. The input data for the simulations of evaluation of the exposure with consumption, that is,number of users (n), average, minimum and maximum and distribution function of best fit are presented in Table 1.

High demand foods	Ingredients	Pathogens detected	Risk analysis
Chicken	raw chicken meat	*Salmonella enterica* (40%)	No cooking involved, bad manufacturing practices during marketing and distribution, and improper storage temperature do not provide sufficient protection from pathogens in fresh meat.
Groundbeef	Raw beef	*Escherichia coli* (40%) *Salmonella enterica (10%)*	No cooking is involved, lousy manufacturing practices during marketing and distribution, and improper storage temperature do not provide sufficient protection from pathogens in fresh beef.
Freshcheese	Unaged cheese made from pasteurized or boiled milk	*Escherichia coli* (60%), *Listeria monocytogenes* (27%), Salmonella enterica (15%)	Unpasteurized milk, consuming fresh cheese,sold in outdoor markets
Bolón	Mashed green plantains with cheese and pork,baked or fried	*Escherichia coli* (20%) *Listeria monocytogenes* (17%) (25%)	Products are made by hand, not stored in refrigeration,and the rotation concerning the sale depends onthe consumption of the customers
Ceviche	Cooked fish with raw onions, peppers, tomatoes, and cilantro marinated in lemon juice	*Escherichia coli* (20%)Salmonella enterica (10%)	Products made by pickling fish without heat treatment and dressings are not adequately disinfected.
Encebollado	Cooked fish and cassava soup withraw onions and cilantro	Escherichia coli (80%) Salmonella enterica (10%)	Dressings used in the preparation are not adequatelydisinfected, and no heat treatment is applied.
Sauces	Homemade mayonnaise and other tomato- and onion-based raw sauces	Salmonella enterica (20%), Escherichia coli (20 to 40%)	Homemade without good manufacturing practices
Fruits	Strawberries, grapes	*Escherichia coli* (40%)	Inadequate cleaning and disinfection
Fruit juice	Fresh homemade orange juice or lemonade with no thermal treatment	*Escherichia coli* (40%) Listeria monocytogenes (15%)	Inadequate cleaning and disinfection
Fruit salad	**Mixed cut fruit with no thermal treatment**	**Escherichia coli (80%), Listeria monocytogenes (80%)**	Prepared unhygienic conditions, contaminated in-igredients

Table 1. Hazards in Ecuador demand food products

Dose-response relationship

Listeria monocytogenes was detected in 27% of the unpasteurized fresh cheese samples and 80% of fruit salad. *Salmonella spp.* was detected in 40% of the samples of raw chicken meat and 20% of homemade sauces, and *Escherichia coli* was detected in 60% of unpasteurized fresh cheese samples, 20% in bolón and ceviche, 80% in Encebollado, from 20 to 40% in artisanally prepared sauces, 40% in ready-to-eat fruits such as strawberries and grapes, 40% in fruit juices and 80% in fruit salad. The probability distribution through the dose-response graph shown in Figure 1 demonstrates the possibility of *E. coli infection* in 9 types of food consumed by people from Guayaquil, Quito and Cuenca between 18 and 46 years of age. Of 96.8% sauces with P50-P99: 0.0003-0.0023 log $_{10}$ cfu/serving, ground meat 94.6% with P50-P99:0.0013-0.0122 log $_{10}$ cfu/serving, fresh cheese 96.2% with P50-P99:0.0003-0.0041 log $_{10}$ cfu/serving, encebollado 96.7% with P50-P99:0.0036- 0.0282 logs $_{10}$ cfu/serving, ceviche 96.2% with P50-P99:0.0036-0.0297 log $_{10}$ cfu/serving, bolón 95.7% with P50-P99:0.0013-0.0116 log $_{10}$ cfu/serving, fruit salad 93.2% with P50 - P99:0.0019-0.0199 log $_{10}$ cfu/serving, ready-to-eat fruit 96.8% with P50-P99:0.0014-0.0144 log $_{10}$ cfu/serving and fruit juice92.4% with P50-P99: 0.0030 -0.0299. The data obtained in the present study for the risk of infection of ground meat are slightly similar to those reported by [27] in meat products consumed in Qatar.

The QMRA simulations show a higher probability of the risk of *E. coli infection due* to the consumption of sauces and ready-to-eat fruits than the rest of the foods evaluated.

The dose of *Escherichia coli* per serving of foods such as fresh cheese and sauces was lower concerning bolón, ground meat, fruit salad, fruit, fruit juice, Encebollado and ceviche, as shown in Table 4.

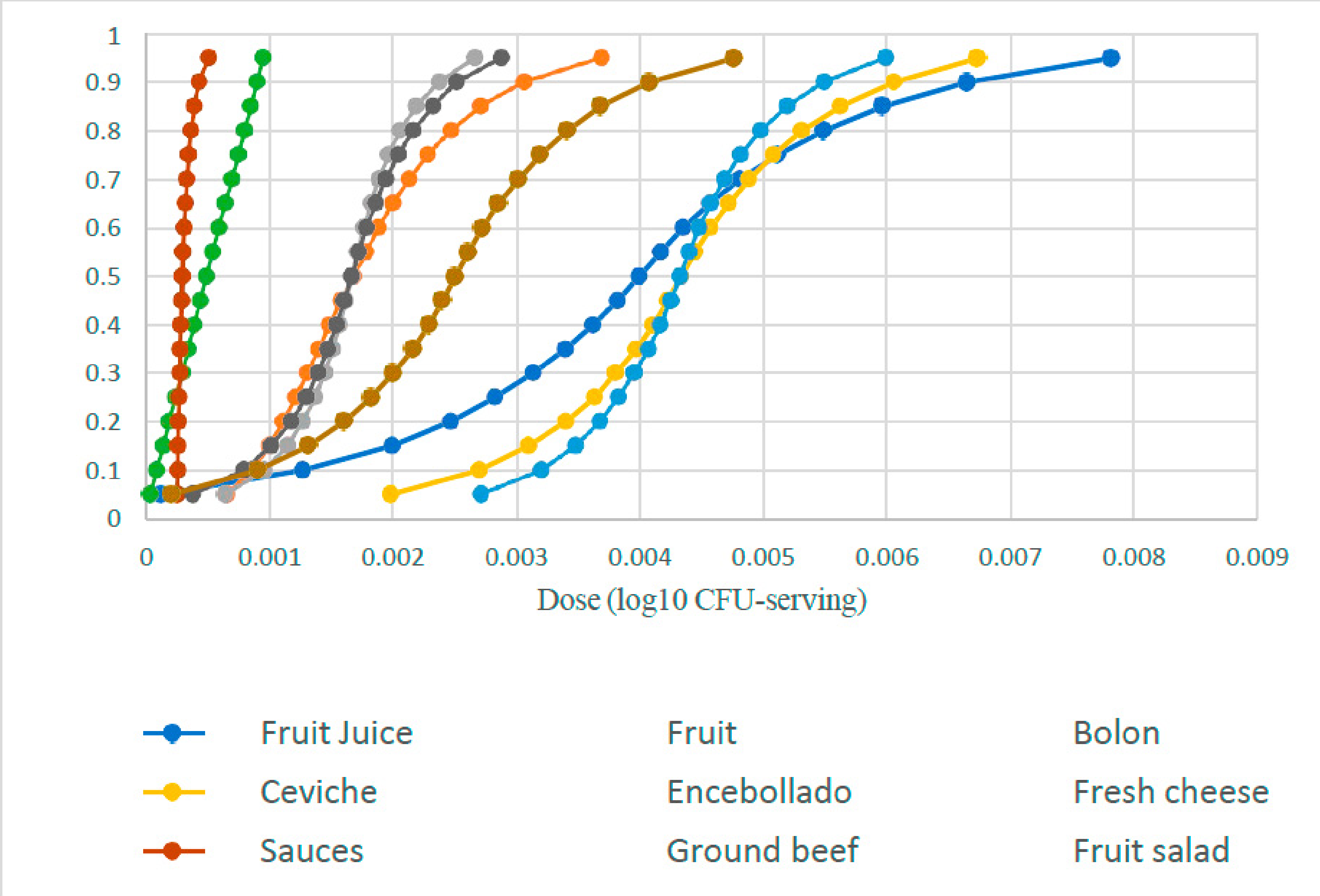

Figure 1: Dose-response graph for *E.coli* in 9 types of food evaluated in Guayaquil, Quito, Cuenca

Type of food	Mean	Std deviation	50% Perc	75% Perc	90% Perc	95% Perc	97.5% Perc	99% Perc	% risk
Ground beef	0.0022	0.0027	0.0013	0.0030	0.0054	0.0073	0.0094	0.0122	**94.6%**
Sauces	0.0004	0.0005	0.0003	0.0006	0.0010	0.0013	0.0017	0.0023	**96.8%**
Fresh cheese	0.0006	0.0009	0.0003	0.0008	0.0016	0.0024	0.0031	0.0041	**96.2%**
Encebollado	0.0056	0.0060	0.0036	0.0078	0.0134	0.0176	0.0218	0.0282	**96.7%**
Ceviche	0.0056	0.0064	0.0036	0.0077	0.0135	0.0179	0.0227	0.0297	**96.2%**
Bolón	0.0022	0.0025	0.0013	0.0029	0.0053	0.0071	0.0090	0.0116	**95.7%**
Fruit salad	0.0033	0.0043	0.0019	0.0045	0.0084	0.0114	0.0150	0.0199	**93.2%**
Fruits	0.0024	0.0031	0.0014	0.0031	0.0059	0.0080	0.0105	0.0144	**96.8%**
Fruit juice	**0.0052**	**0.0069**	**0.0030**	**0.0071**	**0.0133**	**0.0183**	**0.0234**	**0.0299**	**92.4%**

Table 4: Outputs of the QMRA model that represent the probability of infection by *E coli* per portion of food evaluated in Guayaquil, Quito and Cuenca, all expressed as log 10 CFU /per serving

Figure 2 demonstrates the possibility of *Salmonella enterica infection* in 7 types of food, consumption of 100% sauces with P50-P99: 0.0000235-0.0000582 log $_{10}$ cfu/serving, ground meat 97.7% with P50 - P99:0.000137-0.0003005 log $_{10}$ cfu/serving, 100% chicken with P50-P99:0.0001380- 0.0003430 log $_{10}$ cfu/serving, fresh cheese 99.5% with P50-P99:0, 0000407-0.0000796 log $_{10}$ cfu/serving, 99.9% encebollado with P50-P99:0.0003461-0.00057 log $_{10}$ cfu/serving, ceviche 99% with P50-P99:0.0003471-0.0006593 log $_{10}$ cfu/serving, bolón 98.9% with P50-P99:0.0001337- 0.0002651log $_{10}$ cfu/serving. These values are similar to those reported by [28], a study onready-to-eat vegetables. The QMRA shows a higher probability of *Salmonella* infection in the combination of sauces and chicken than in the other evaluated foods.

The dose of *Salmonella enterica,* such as fresh cheese and sauces, was lower in bolón, ground meat, chicken, Encebollado, and ceviche, as shown in Table 5.

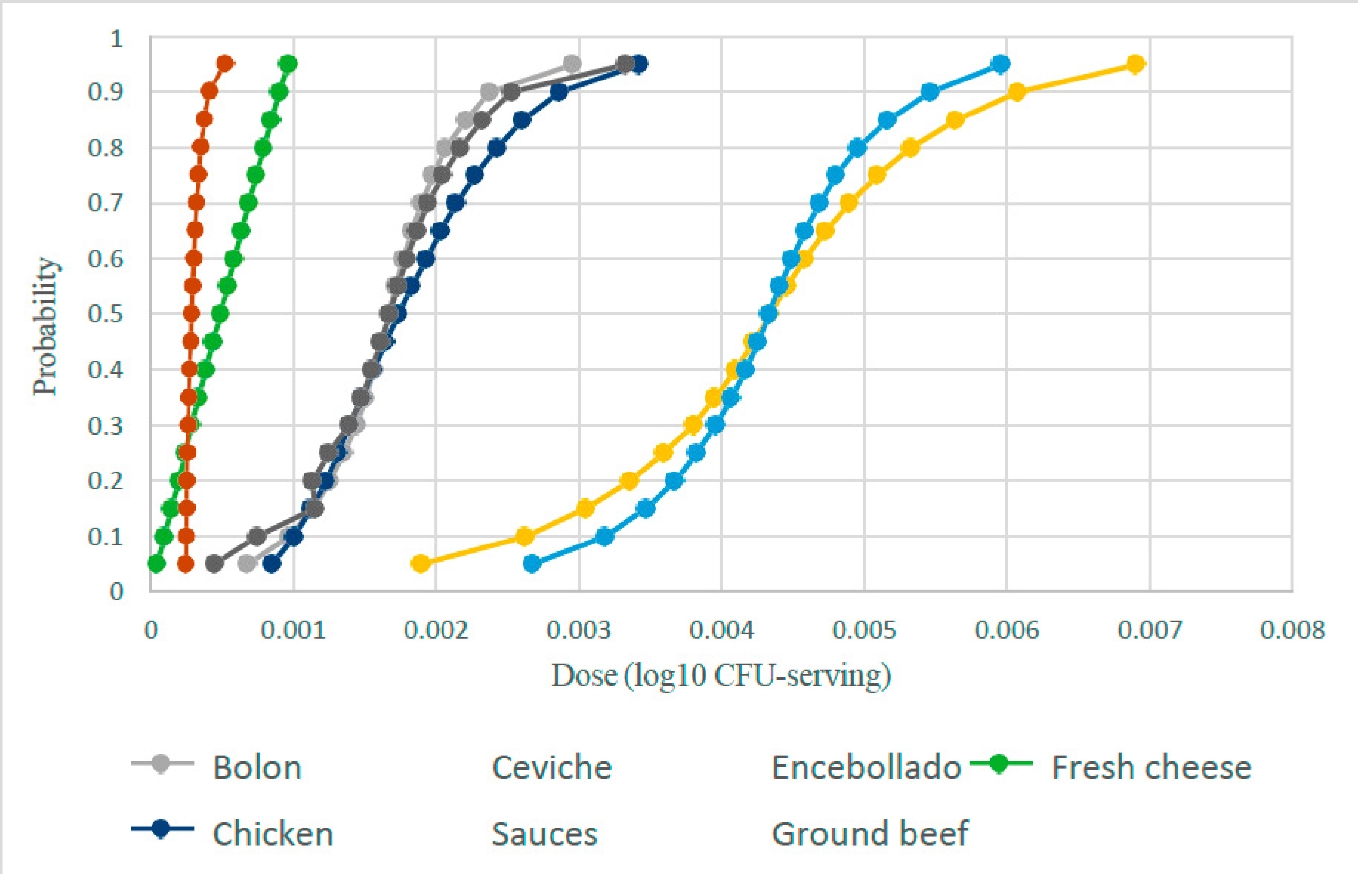

Figure 2: Dose-response graph for *Salmonella* in 7 types of food evaluated in Guayaquil, Quito,Cuenca

Type of food	Mean	Std deviation	50% Perc	75% Perc	90% Perc	95% Perc	97.5% Perc	99% Perc	% Risk
Ground beef	0.0001333	0.0000623	0.0001337	0.0001632	0.0002057	0.0002345	0.0002647	0.0003005	97.7%
Sauces	0.0000261	0.0000083	0.0000235	0.0000277	0.0000340	0.0000404	0.0000467	0.0000582	100.0%
Chicken	0.0001478	0.0000616	0.0001380	0.0001798	0.0002282	0.0002643	0.0002982	0.0003430	100.0%
Fresh cheese	0.0000404	0.0000233	0.0000407	0.0000605	0.0000727	0.0000765	0.0000784	0.0000796	99.5%
Encebollado	0.0003460	0.0000802	0.0003461	0.0003859	0.0004361	0.0004788	0.0005183	0.0005700	99.9%
Ceviche	0.0003467	0.0001184	0.0003471	0.0004046	0.0004814	0.0005395	0.0005945	0.0006593	99.0%
Bolón	0.0001335	0.0000483	0.0001337	0.0001571	0.0001883	0.0002128	0.0002345	0.0002651	98.9%

Table 5: Outputs of the QMRA model that represent the probability of infection by *Salmonella* per portion of food evaluated in Guayaquil, Quito and Cuenca, all expressed as log 10 Ufc per serving

Figure 3 shows the probability of *Listeria monocytogenes infection* in 4 types of food, fresh cheese99.6% with P50-P99:0.0000586-0.0001193 log10ufc/serving, bolón 98.9% with P50-P99:0 .0002003-0.0004015 log $_{10}$ cfu/serving, fruit salad 95.7% with P50-P99:0.0002986- 0.0007436 log $_{10}$ cfu/serving, and fruit juice 95.5% with P50-P99: 0.0004773 -0.0012412 log $_{10}$ cfu/serving. The values presented concerning the concentration of *Listeria* per portion of food in the present study concerning the possibility of infection are similar to those reported by [28]. The QMRA shows a higher probability of infection by *Listeria monocytogenes* in consuming unpasteurized fresh cheese and bolón than in foods such as salads and fruit juices.

The doses of *Listeria monocytogenes* per serving of food observed for fresh cheese were lower with bolón, fruit salad and fruit juices, as shown in Table 6.

Figure 3: Dose-response graph for *Listeria monocytogenes* in 4 types of food evaluated in Guayaquil,Quito, Cuenca

Type of food	Mean	Std deviation	50% Perc	75% Perc	90% Perc	95% Perc	97.5% Perc	99% Perc	% risk
Fresh cheese	0.0000592	0.0000347	0.0000586	0.0000887	0.0001082	0.0001145	0.0001175	0.0001193	**99.6%**
Bolón	0.0002004	0.0000742	0.0002003	0.0002371	0.0002853	0.0003192	0.0003577	0.0004015	**98.9%**
fruit salad	0.0002980	0.0001678	0.0002986	0.0003816	0.0004905	0.0005708	0.0006497	0.0007436	**95.7%**
fruit juice	**0.0004779**	**0.0002842**	**0.0004773**	**0.0006180**	**0.0007993**	**0.0009376**	**0.0010716**	**0.0012412**	**95.7%**

Table 6: Outputs of the QMRA model that represent the probability of infection by *Listeria monocytogenes* per portion of food evaluated in Guayaquil, Quito and Cuenca, all expressed as log 10 CFU per serving

Evaluation of different scenarios based on the probability of infection based on a single dose.

The QMRA model was used to simulate the risk of infection in *E coli* in foods such as ground beef, homemade sauces, unpasteurized fresh cheese, bolón, Encebollado, ceviche, ready-to-eat fruits, fruit juice and fruit salads, *Salmonella enterica* in foods such as chicken, ground beef, unpasteurized fresh cheese, homemade sauces, bolón, ceviche and Encebollado *and Listeria monocytogenes* in unpasteurized fresh cheese, bolón, juice and fruit salad.

To characterize the risk and determine the probability of getting sick based on a single dose, the exponential dose-response model was used in a study carried out by [21] [22], applying r values for *Salmonella, Listeria monocytogenes and E coli* described by [23] [21] [24], respectively and D is the dose consumed (number of cells per slice).

The data of the consumed dose (D) for the characterization of the risk of each one of the pathogens evaluated in model [21] [22] were averaged about P5 - P99 obtained from the Monte Carlo simulation (100,000 iterations) using the @RISK 7.5 software (PALISADE Cooperation) which isa plugin for Microsoft Excel, Table 7 shows the probability of getting sick for *Listeria, Salmonella and E. coli.* In the case of ground meat contaminated with *E. coli*, it is evident that there is a probability that a person will get sick for every 6 portions consumed; for sauces, one case of illness for every 33 contaminated portions consumed; for fresh cheese, one case of illness for every 25 contaminated portions consumed, Encebollado and ceviche one case of disease for every 3 contaminated portions consumed, for bolón one case of disease for every 3 contaminated portions consumed, for fruit salad one case of disease for every 4 contaminated portions consumed, for fruit 1 case of illness for 3 contaminated portions consumed and fruit juices 1 case of illness for every 3 contaminated portionsconsumed.

For the evaluation of *Listeria monocytogenes,* there is no evidence of disease risk per portion consumed of foods evaluated, such as bolón, cheese, salad and fruit juices. This could be due to thelow prevalence (2%) of *Listeria monocytogenes;* since it is a highly psychotropic microorganism, the cold chain seems to contribute significantly to mitigating the risks associated with this pathogen [28].

Name	**Ground beef**	**Sauces**	**Fresh cheese**	**Encebollado**	**Ceviche**	**Bolón**	**Fruit salad**	**Fruits**	**Fruit juice**
Food size (g)	150	30	40	430	430	340	280	430	**480**
Probability of *E.coli infection*	1.5600E-01	3.33557E-02	0.0442428	0.3878459	0.3878598	0.3200084	0.2725639	0.3861008	**0.4215437**
% Probability	16	3	4	39	39	32	27	39	**42**
Name	**Ground beef**	**Sauces**	**Fresh cheese**	**Encebol-lado**	**Ceviche**	**Bolón**	**Chicken**		
Food size (g)	150	30	40	430	430	340	160		
Probability of *Salmonella enterica*	1	1	1	1	1	1	1		
% Probability	**100**	**100**	**100**	**100**	**100**	**100**	**100**		

Name	**Fresh cheese**	**Bolón**	**Fruit salad**	**Fruit juice**					
Food size (g)	40	340	280	480					
Probability of Listeria infection	9.50E-13	8.09E-12	6.67E-12	1.15E-11					
% Probability	0	0	0	0					

Table 7: Probability of infection of pathogens in food evaluated by single dose model Dose expressed in (log10 CFU-serving)

For the evaluation of *Salmonella enterica in foods* such as ground meat, sauces, fresh cheese, encebollado, ceviche, bolón and chicken, the probability that a case of disease will develop for each contaminated portion consumed. The high prevalence of Salmonella enterica in chickens (10.5%), pigs (24.4%) and milk (29.4%) was reported by [29], in fish (94%) and shrimp (100%) [30], maybe oneof the causes of the high risk of acquiring salmonellosis in the evaluated foods, also associated with inadequate handling in their preparation and storage.

Strategies aimed at reducing the prevalence and concentration of *Salmonella* in food preparation, such as proper hygiene and proper storage, while maintaining the cold chain may be more effectivein reducing the risk of this pathogen. Depending on the combination of food and pathogens, the measures chosen will most effectively reduce the risk of infection and the expected number ofcases.

DISCUSSION

This study must address some uncertainties to understand better and interpret the results and their implications.

First, more and more data is needed to improve the accuracy of risk assessment models, including those developed here. Despite this need, the results suggest that Salmonella, the frequently foodborne pathogen with disease outbreaks in Ecuador [31], poses a measurable risk to Ecuadorian products. Our results suggest that more data and strategies should be developed to mitigate *Salmonella* in food in Ecuador.

This model could help evaluate specific situations of microbiological contamination in food, such as foodborne illness outbreaks or focused contamination events[1]. It allows you to analyze risk events in detail, identify underlying causes and develop mitigation and prevention strategies. This approach could provide relevant information on crisis management and response to contamination situations in the context of the cases studied.

Salmonella contamination was not detected in foods such as ready-to-eat fruits, salads, and fruitjuices, as well as *Escherichia* coli in chickens and *Listeria monocytes* in chicken, ground beef, ready-to-eat fruits, Encebollado, ceviche, and sauces due to the place of origin sampling and numberof samples selected which could be a negative bias in the evaluation of risk analysis.

Another source of uncertainty is determining disease cases in the exposed population (total cases per 100,000 inhabitants). Information on the percentage of consumers (general population and risk) of the food concerning the total population must be available to improve the modeling of the risk characterization.

The study evaluating microbiological risks in foods in high demand in Ecuador found that the percentage of pathogens varied depending on the type of food evaluated. It was observed that 96.8% of the sauces, 94.6% of the ground meat, 96.2% of the fresh cheeses, 96.7% of the onion, 96.2% of the ceviche, 95.7% of the bolón, 93.2% of the salad of fruits, 96.8% of ready-to-eat fruits and 92.4% of fruit juices had the presence of pathogens in different concentrations. The pathogens found in the microbiological risk assessment study in high-demand foods in Ecuador included Salmonella, Escherichia coli and Listeria monocytogenes.

According to Regulation (EC) 2073/2005[2] for Salmonella, foods must not contain Salmonella as it is a pathogen that can cause severe infections and, therefore, any presence of Salmonella in foods is unacceptable. Likewise, for *E. coli* similar to Salmonella, foods must meet food safety criteria for E. coli to ensure they are safe for human consumption. Although many strains of E. coli are harmless, some, such as E. coli O157, can cause serious illness. E. coli in food indicates possible fecal contamination and a health risk. Finally, for Listeria monocytogenes, ready-to-eat foods must not exceed the limit of 100 CFU/g (colony forming units per gram) of Listeria monocytogenes during their shelf life. This limit is set to minimize the risk of listeriosis, a severe infection that can be especially dangerous for pregnant women, newborns, the elderly, and people with weakened immune systems.

The amount of Listeria monocytogenes found in the food samples evaluated in the Ecuador study exceeds the permitted limit of 100 CFU/g established by the regulations for ready-to-eat foods, so it is considered outside the acceptance range according to the rule. Salmonella was not detected in certain foods such as ready-to-eat fruits, salads, and fruit juices, nor was Escherichia coli in chickens or Listeria monocytogenes in several foods mentioned [3]. However, the importance of considering possible biases in risk assessment due to sample selection and sampling locations is highlighted.

The Quantitative Microbiological Risk Assessment (QMRA) model used in the study allowed us to estimate the probability of Listeria monocytogenes infection per portion of food in Guayaquil, Quito and Cuenca[4]. The results obtained were expressed as log10 CFU (Colony Forming Units) per portion, which provides information on the microbial load present in each portion of food and, therefore, the risk associated with consuming contaminated food. To interpret these results more clearly, it is essential to understand that log10 CFU per serving represents the amount of Listeria monocytogenes present in a specific serving of food. The higher the log10 CFU value, the greater the microbial load and, therefore, the greater the risk of infection if that contaminated portion is consumed.

If a result of 0.0001478 log10 CFU per serving of chicken is obtained in Guayaquil, it means that there is a certain amount of Listeria monocytogenes present in that serving of chicken, indicating a potential risk of infection if consumed. By comparing these results between different food types and cities, the relative risk of Listeria monocytogenes infection associated with the consumption of each food in different locations can be assessed and compared.

The Monte Carlo simulation results in the study provided detailed estimates on the probability of infection from consuming foods contaminated with Salmonella, Listeria monocytogenes and Escherichia coli in Ecuador; specific results obtained from the Monte Carlo simulation included:

For Salmonella: Simulations were carried out to estimate the probability of Salmonella infection from consuming foods such as ground beef, chicken, unpasteurized fresh cheese, homemade sauces, bolón, ceviche and onion[5].

Probabilistic distributions were obtained that reflected the variability in the probability of Salmonella infection depending on the pathogen concentration in food and food consumption.

For Listeria monocytogenes: Simulations were carried out to estimate the probability of Listeria monocytogenes infection from consuming foods such as unpasteurized fresh cheese, bolón, fruit juice, and fruit salad[5].

Probabilistic distributions were generated, representing the variability in the probability of Listeria monocytogenes infection concerning the pathogen concentration in the food and the amount consumed.

For Escherichia coli: Simulations were performed to estimate the probability of Escherichia coli infection from the consumption of foods such as ground meat, homemade sauces, unpasteurized fresh cheese, bolón, Encebollado, ceviche, ready-to-eat fruits, fruit juice, and fruit salad[6].

Probabilistic distributions were generated, showing the variability in the probability of Escherichia coli infection as a function of the pathogen concentration in the food and the amount consumed.

The simulation estimated the probability of Salmonella infection from consuming foods such as ground beef, chicken, unpasteurized fresh cheese, homemade sauces, bolón, ceviche, and onion. Probabilistic distributions were obtained that reflected the variability in the probability of Salmonella infection depending on the pathogen concentration in food and food consumption[7].

The simulation estimated the probability of Listeria monocytogenes infection from consuming foods such as unpasteurized fresh cheese, bolón, fruit juice, and fruit salad. Probabilistic distributions were generated, representing the variability in the probability of Listeria monocytogenes infection regarding the pathogen concentration in the food and the amount consumed. For Escherichia coli: The simulation estimated the probability of Escherichia coli infection from the consumption of foods such as ground meat, homemade sauces, unpasteurized fresh cheese, bolón, Encebollado, ceviche, ready-to-eat fruits, fruit juice, and fruit salad.

Probabilistic distributions were generated, showing the variability in the probability of Escherichia coli infection as a function of the pathogen concentration in the food and the amount consumed.

CONCLUSIONS

This study is the first risk analysis evaluation report concerning the consumption of high-demand foods in the cities of Guayaquil, Quito and Cuenca associated with pathogens such as *Escherichia coli, Listeria monocytogenes* and *Salmonella.*

The presence of *Salmonella* was more relevant concerning the probability of getting sick per portion of contaminated food compared to *E.coli*, in which the risk of getting sick s lower, and *Listeria monocytogenes*, where there was no evidence of risk or probability of disease. The dose-response model obtained by simulating the data from the @risk software shows that for *E.coli* and *Salmonella,* a small portion of ingested contaminated food could pose a risk of infection, just like unpasteurized fresh cheeses in *Listeria*, due to the size of the portion associated with the food concerning consumption per person.

The development of risk management actions for more excellent monitoring and mitigation of contamination by *Salmonella*, *Listeria* and *E.coli* in high-demand foods such as those estimated in this study that are highly consumed in our country could lead to the protection of consumers in generalin the field of food safety, especially in vulnerable populations such as children, pregnant womenand older adults with compromised immune systems.

However, it is essential to conduct follow-up studies to measure exposure levels under otherfood processing and preparation conditions that may pose a significant food safety concern.

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Additional information Correspondence should be addressed to *ejsalazar@utb.edu.ec*
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