

Salmonella in Chicken and Pork Meat as a Likely Major Contributor to Foodborne Illness in Peru

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Abstract. Nontyphoidal *Salmonella* is one of the major causes of self-limiting diarrheal disease and the most common foodborne pathogen worldwide. It is an important contributor to the burden of foodborne illness in South America, including Peru, where chicken and pork are important vehicles for *Salmonella* infection. *Salmonella* infections are under-reported, particularly in low- and middle-income countries where concerted action tackling *Salmonella* along the chicken and pork chains, from primary production to retail, is urgently needed. To support and inform the implementation of new strategies to reduce *Salmonella* contamination of chicken and pork, this study describes the frequency and distribution of foodborne outbreaks attributed to *Salmonella* in Peru and evaluates the level of *Salmonella* in chicken and pork meat sold in markets of three regions of Peru. To that end, we analyzed historical reports of foodborne outbreaks, levels of *Salmonella* in chicken and pork sold in markets, and the number of mesophiles in the collected meat samples. As a result, the microbiological analysis reveals a widespread contamination of chicken (77.1%) and pork (26.8%) with *Salmonella*. It also pinpoints *Salmonella* as the causative agent in nearly half of the outbreaks (47.0%) where the potential origin is identified over a 11-year period with chicken, mayonnaise, and pork being the most likely food vehicles. These results suggest that *Salmonella* is a major contributor to foodborne illness in Peru and that the monitoring of mesophiles could be a good strategy for surveillance, generating data to support source attribution studies and ultimately evidence-informed policies.

INTRODUCTION

The WHO estimates that every year one in 10 people suffers foodborne illness, causing 33 million healthy life years lost and 420,000 deaths, especially in children under 5 years old (one in three deaths are because of foodborne diseases).^{1,2} In 2020, salmonellosis was the second most reported foodborne illness in the European Union, with more than 52,700 cases reported³; and in the United States, *Salmonella* has been estimated to cause 1.35 million cases per year,⁴ mostly as a result of consumption of chicken and turkey.⁵ However, the latest report of the Interagency Food Safety Analytics Collaboration⁶ estimated that more than 75% of *Salmonella* illnesses were attributed to seven food categories, with approximately 40% of cases attributed to meat products (chicken 17.3%, pork 12.8%, beef 6%, and turkey 5.9%) and no statistically significant difference between chicken 17.3% (90% CI: 13.6–21.6%), fruits 14.9% (90% CI: 11.1–19.4%), and seeded vegetables 12.0% (90% CI: 8.2–16.5%). More recent reports from the United States estimate a major role of nonfood transmission pathways such as waterborne, person-to-person, animal contact, and environmental, and the proportion of domestically Salmonellosis acquired through food was reduced from 94% in 2011⁷ to 66% in 2021.⁸

Salmonella spp. may be found in the intestinal tract of chickens and pigs; hence, the carcasses and subsequently

the meat products can be accidentally contaminated from fecal material during slaughtering.^{9,10} Importantly, contamination of food can also arise at later stages of the food chain through unhygienic handling or cross-contamination of food from surfaces not properly disinfected.¹¹ *Salmonella* spp. can grow in a wide range of temperatures, from 6 to 45°C and although the optimal temperature for growth is ~37°C, the minimum infective dose is low, believed to range between 7 and 36 colony forming units.¹² Hence, even if growth is prevented at refrigeration temperature, the presence of a small amount of bacteria in raw meat is enough to potentially cause infection if meat is not properly cooked.¹³ Reducing *Salmonella* infections resulting from consumption of food, particularly poultry, has therefore become a priority for many countries including the United States and countries in the European Union.¹⁴

In low and middle-income countries (LMICs), including most countries in Latin America, data on the incidence of foodborne illness are scarce. However, it is widely accepted that the incidence of foodborne illness in these countries is likely to be much higher than in high-income countries.^{15,16} In Peru, according to the National Center for Disease Epidemiology, Prevention and Control (CDC MINSa), an average of 47 annual outbreaks of foodborne diseases were reported between 2014 and 2018, involving an estimated 6,098 people and resulting in 1,311 hospitalization and 29 deaths throughout the country.¹⁷ In 2019, one in five cases of foodborne disease was attributed to *Salmonella* spp., however, it should be noted that cases of gastrointestinal disease are often self-limiting and resolved without resorting to public health services. Therefore, the reported disease events are likely to represent only a fraction of the total disease burden

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in the population.¹⁶ Information about outbreaks is scarce and a more detailed analysis is needed to carry out a thorough investigation; thus, we compiled the Peruvian public health authorities' reports from January 2010 to January 2021.

In many of LMICs, availability of reliable epidemiological or microbiological data is often the main barrier to systematic identification of food safety priorities,¹⁸ and this is true for Peru and Latin America in general. Despite efforts to identify the causative agent of foodborne diseases through the national network of laboratories of the National Institutes of Health of Peru, only 13 strains of *Salmonella* spp. were isolated through the food safety surveillance system between 2003 and 2007.¹⁹ This substantial lack of information on the cases attributed to *Salmonella* spp. in Peru highlights the need for quality data to allow not only a more accurate estimation of the national burden of foodborne diseases but also to support a scientific-based food safety policy.

As in other LMICs, in Peru, traditional food markets represent a key component of the food system, playing important economic and sociocultural roles.^{20–22} Implementation of food controls in these markets presents challenges given the complex network of diverse actors involved in production, transformation, distribution, and retailing of the different food products channeled through them. However, it is widely recognized that traditional food markets can make a substantial contribution to livelihoods in LMICs by promoting local, sustainable, and equitable food chains.²³ Furthermore, a large proportion of the population source their foods from these markets. In Peru, according to the 2021 National Household Survey,²⁴ 70% of consumers purchase pork and chicken meat in traditional food markets. It is therefore critical to characterize the food safety challenges associated with these markets to enable the design and implementation of realistic yet effective risk mitigation strategies and controls.²⁵

To provide valuable data for the development of future source attribution studies on *Salmonella* spp. given that pathogens that may be playing an important role in the appearance of foodborne outbreaks in Peru, the aims of the study were as follows: 1) to describe the frequency and spatiotemporal distribution of foodborne outbreaks attributed to *Salmonella* spp. reported by the public health authorities in Peru and 2) to assess the status, with respect to *Salmonella*, of chicken and pork meat on retail in traditional food markets in several locations in Peru. To that end, we analyzed secondary data from foodborne disease surveillance and microbiological data generated by studying chicken and pork products for retail sale.

MATERIALS AND METHODS

Overview of the analytical approach. The study includes two components: 1) a retrospective analysis of foodborne surveillance data over a period of 11 years in Peru and 2) an assessment of the microbiological status (concentration and presence/absence of mesophiles and *Salmonella* spp.) of chicken and pork meat on sale in traditional food markets in three distinct geographic areas in Peru. The first component relied on data from officially reported foodborne outbreaks between 2010 and 2021. These data were used to summarize the characteristics of outbreaks in which *Salmonella*

spp. was identified as the likely causative agent and to assess whether outbreaks linked to *Salmonella* spp. exhibited spatiotemporal clustering. The second component involved collection of pork and chicken samples from six markets in three distinct geographic areas of Peru and testing of those samples for the presence (and amount) of mesophiles and *Salmonella* spp. For this study, we consider three contrasting cities (with different climates) that capture to some extent the commercial flows in the country: 1) Huancayo, a city in the central highlands of Peru at 3,271 m above sea level that represents the commercial hub of the central Peruvian Andes with a typical mountain climate (the average temperature in Huancayo ranges from 9.5 to 12.5°C with minimum and maximum temperatures of approximately –5 and 28°C); 2) Huaral, an important supply center for the capital (Lima), located in the Peruvian coast and in the proximity of Lima, with a temperate climate (the temperature usually varies from 16 to 28°C and rarely drops below 14°C or rises above 30°C); and 3) Tumbes, also on the Peruvian coast but farther from Lima, with a typical hot tropical climate and a mean temperature ranging between 27 and 31°C throughout the year.

Finally, the correlation between mesophile and *Salmonella* counts was assessed and the presence of *Salmonella* compared across species (pork versus chicken), locations (three regions) and markets (with suboptimal versus adequate hygiene). Using a Bayesian framework, credible intervals were obtained for the probability of a sample of chicken or pork being contaminated by *Salmonella* across locations and types of markets.

Retrospective analysis of foodborne surveillance data.

Data sources and data extraction. A compilation of all the officially reported foodborne outbreaks between 2010 and 2021 was obtained from the CDC-MINSA. This institution receives and registers outbreaks reports from health centers across the country, including the location of the outbreak (district, province, and department), notification date, classification and diagnosis of the event, causative etiologic agent, number of affected people, number of hospitalizations, and number of deceased.

STATISTICAL ANALYSES

Descriptive statistics were obtained for foodborne outbreaks in which *Salmonella* spp. had been confirmed, either as the only hazard involved or together with other foodborne hazards. Data summarized included numbers ill, hospitalized and deaths and the foods that patients reported having consumed. Date of notification of the outbreak and coordinates of the centroid of the district where it was reported (third level administrative divisions in Peru after regions and provinces) were used to assess spatiotemporal clustering of *Salmonella* outbreaks between January 2010 and January 2021 by means of the space–time permutation scan statistic, implemented using retrospective space–time permutation analysis, with time aggregation at the level of the month and 999 permutations in SaTScan version 9.6 (Information Management Services Inc., Calverton, MD). This approach has the advantage of assuming minimal information such as time, geographic location, and outbreak size with no need of population-at-risk data.²⁶

Microbiological status of chicken and pork meat on sale in traditional food markets.

General description of sampled stalls. Structure and setup varied across markets; some are built with solid materials, and stalls are organized according to the type of meat sold, whereas in others, the structure is improvised and changing—specifically, due to the COVID-19 pandemic, improvised stalls were devised using objects such as a cart. In addition, the dynamics of the stalls were frequently changed because there was not much stability during that period, so the stalls could close quickly, not open again, change owners, or go on to sell another type of food.

In the markets that were included in this study, most stalls sell meat from one species only, except for a few stalls in one market in Huancayo and one market in Huaral. From observations of the person collecting samples, chopping boards were mainly made of wood (3 to 4 inches thick) or polypropylene, and in most cases, the level of cleanliness was judged to be poor. Cloths used to clean surfaces were generally old. Although most stalls had a refrigerator, it was difficult to ascertain whether it was working properly; in fact, only two stalls (one in Tumbes and one in Huancayo) had a thermometer with temperature visible to the customers. In most stalls, the meat was hanging or on trays without protection or covering. None of the stalls had flytraps, and flies were observed on the meat in Tumbes and Huaral but not in Huancayo. This may be related to the location (Tumbes and Huaral are in the coast and Huancayo in the mountains) and temperature at the time of sampling.

Sampling. The number of samples to be tested was initially established based on logistical and financial constraints, with 150 stratified into 90 and 60 chicken and pork samples, respectively. These would be obtained from the same number of market stalls (one sample per stall) in the three locations (Huancayo, Huaral, and Tumbes). At each location, two markets were identified based on convenience and feasibility of sample collection. In this manner, the target number of samples would be equally distributed across the six markets in the three locations as follows: Huancayo (markets A and B), Huaral (markets C and D), and Tumbes (markets E and F). Thus, 15 chicken and 10 pork samples would be collected per market, with 30 chicken and 20 pork samples per location (Table 1).

However, the sample collection was carried out between May and October 2021 and required departures from the initial plan due to regulations imposed by the Central Government during the SARS-CoV-2 pandemic. These regulations severely affected market sellers in Peru resulting in fewer than expected stalls selling chicken and pork meat than anticipated according to the information provided in the

census of food markets.²⁷ Because of this, seventy poultry and forty-one pork meat samples were collected with a distribution by market and location presented in Table 1. Briefly, 29 chicken and 20 pork samples were obtained from Huancayo, 30 and 19 from Huaral, and 10 chicken samples from Tumbes.

Markets were visited in the early morning for several weeks, and one sample was taken from all open stalls on the day of the visit. From the second visit onward, samples were only collected from stalls that had not been sampled before.

Fresh neck and chest skin samples were preferred because these are the sample types where microbial contamination is more likely to be found.²⁸ If not available fresh, frozen samples or other cuts were selected. The meat is normally sold in cuts, and depending on the cut or piece to be purchased, further manipulation and chopping is done by the vendor. Type of meat cut and manipulation of the samples were registered.

The meat samples, as received from the vendors, were placed in coolers with ice packs. The temperature was monitored throughout transport, ensuring it remained between 2 and 8°C. Samples were analyzed immediately upon arrival in the laboratory and no later than 24 hours after purchase.

Microbiological analysis.

Mesophiles enumeration. The samples were analyzed according to the ISO 4833-1:2013 for *Mesophiles* count by the pour plate technique. Briefly, 25 g of sample were aseptically weighted and mixed with 225 mL of buffered peptone water (#107228, Merck Millipore, Darmstadt, Germany) to obtain an initial dilution of 10^{-1} . A 10-fold serial dilution, 10^{-1} to 10^{-8} , was prepared, and 1 mL of each dilution was added into sterile petri dishes in duplicate, then 15 mL of plate count agar (#247940, BD Diagnostic, Oxford, United Kingdom) were carefully poured into each petri dish and gently mixed in a circular motion. All the petri dishes were incubated at 30°C for 72 hours, then those plates containing between 15 and 300 colony-forming units (CFU) were counted. Results obtained through quantification were expressed in log₁₀ CFU per gram of sample (log₁₀ CFU/g).

Detection and enumeration of Salmonella spp. The samples were analyzed according to the ISO 6579-1:2017 and ISO 6579-2:2012 for detection and enumeration of *Salmonella* spp. In summary, an initial suspension (10^{-1}) was made by aseptically weighing 25 g of sample and mixed with 225 mL of buffered peptone water (#107228, Merck Millipore). Then, for the pre-enrichment step in a nonselective liquid medium, 2.5 mL of the initial suspension were poured into the first empty column of a 12-well microtiter plate. Serial dilutions were then made (5^{-1} , 5^{-2} , and 5^{-3}) from the initial column by adding 0.5 mL of it to a well

TABLE 1
Comparative table between the initial and actual distribution of samples collected during the study

Geographic Location	Market of Procedure	Initial No. of Samples to Collect		Actual No. of Samples Collected	
		Chicken	Pork	Chicken	Pork
Huancayo	Market A	15	10	19	13
	Market B	15	10	10	7
Huaral	Market C	15	10	28	14
	Market D	15	10	2	5
Tumbes	Market E	15	10	6	0
	Market F	15	10	4	0

containing 2 mL of buffered peptone water. The microtiter plate was incubated at 37°C for 18 hours.

The enrichment step on a selective semisolid medium was done by mixing 20 μ L of the pre-enrichment samples into another 12-well microtiter plate containing 2 mL of modified Semisolid Rappaport–Vassiliades (MSRV) agar (#218681, BD Diagnostic) with novobiocin 10 mg/L (# 109874 Merck Millipore) and incubated at 41.5°C for 24 hours. A qualitative test was performed at the same time by adding 100 μ L of the initial dilution previously pre-enriched at 37°C for 18 hours, to a petri dish containing 15 mL of modified MRSV and incubated at 41.5°C for 24 hours.

The suspected wells showing a gray-white, turbid zone extending out from the inoculated drop were selected, and 1 μ L loop was used to inoculate the surface of a xylose lysine desoxycholate (XLD) plate (#CM0469, Oxoid, Basingstoke, United Kingdom) and a Chromogenic *Salmonella* Agar R&F plate (# M1350, R&F Products, Downers Grove, IL), which were incubated at 37°C and 35°C, respectively, for 24 hours. Finally, the suspected well-isolated colonies were selected for biochemical confirmation: TSI-Triple sugar/iron (#CM0277B, Oxoid), urea (#211795, BD Diagnostic), and LDC-L-lysine decarboxylation (#CM0308, Oxoid).

The most probable number (MPN) was obtained by counting the number of positive wells in the four dilutions and in the three repetitions system used as described by the International Organization for Standardization, and the numbers were calculated using the “MPN” R package.²⁹ Results obtained through this method were expressed in log₁₀ most probable number per gram of sample (log₁₀ MPN/g).

Analysis of microbiological data. Given the nature of the data distribution and the Peruvian normative, the results were also analyzed after categorization as follows: for mesophiles data were categorized as “Low” or “High” with a cutoff of 7 log₁₀ CFU/g; for *Salmonella* the counts were categorized into three groups: absence of log₁₀ MPN/g, from 0 to 1.9 log₁₀ MPN/g, and more than 2 log₁₀ MPN/g, with the intention of separating samples with “very high” level of contamination and considering more than 2 log₁₀ MPN/g as a suitable cutoff. The proportion of samples in different categories of *Salmonella* and mesophile contamination by location and market are presented in Tables 2 and 3 as well as by condition and cut.

Fisher’s exact test was used to assess differences between the proportions of samples falling in the categories described earlier between pork and chicken. Wilcoxon signed-rank and Kruskal–Wallis tests were used to assess the univariate associations between mesophile CFU per gram of meat and 1) type of meat (chicken versus pork) and 2) sampling location (Huancayo, Huaral, or Tumbes). To perform all-pairs comparison after significant Kruskal–Wallis test result, Dunn’s all-pairs rank comparison test was used. The hypothesis that *Salmonella* and mesophile counts are associated was assessed by means of Spearman’s rho correlation and χ^2 test for trend.

Given that the normative requires absence of *Salmonella*, assessment of the proportion of samples fulfilling the requirement was warranted. To that end, the probability of presence of *Salmonella* in meat samples from a given market was estimated using a beta-binomial model. The number of samples positive (θ_i) and sampled (n_i) is a random draw from a binomial distribution $y_i \sim \text{bin}(n_i, \theta_i)$, where $\theta_i \sim \text{beta}(\alpha, \beta)$. Generic

TABLE 2

Sample category (very low, low, or high) based on mesophile log₁₀ colony-forming units per gram (log₁₀ CFU/g) of chicken ($N = 69$) and pork ($N = 39$) in samples from six markets in three locations in Peru by individual market, level of contamination, perceived level of hygiene, condition, and type of cut

Location	Market	Chicken Mesophile			Pork Mesophile				
		N	Very Low	Low	High	N	Very Low	Low	High
Huancayo	A	19	0	14	5	13	0	7	6
	B	10	0	7	3	7	0	1	6
Huaral	C	28	0	7	21	14	0	9	5
	D	2	0	2	0	5	0	4	1
Tumbes	E	6	0	0	6	–	–	–	–
	F	4	0	1	3	–	–	–	–
Level of hygiene									
Acceptable		49	0	23	26	32	0	20	12
Suboptimal		20	0	8	12	7	0	1	6
Condition									
Frozen		6	0	1	5	5	0	2	3
Refrigerated		5	0	1	4	18	1	12	5
Fresh		58	0	29	29	16	0	6	10
Neck cut									
No		33	0	12	21	37	1	19	17
Yes		36	0	19	17	2	0	1	1

Very low category has values <5 log₁₀ CFU/g, low category has values between 5 and 7 log₁₀ CFU/g, and high category >7 log₁₀ CFU/g. The pork with very low mesophiles (<5 log₁₀ CFU/g) was included in the low group.

uninformative parameters were used to estimate α, β . The model was implemented in Python 3.9. using PyMC3 3.7.^{30,31} The No-U-Turn Sampler was used to draw 6,000 samples from the joint posterior distribution, discarding the initial 2,000 as tuning and burn-in. The convergence of four Markov chain Monte Carlo chains was visually inspected. Traceplots for these runs are shown in the supplementary material. Gelman–Rubin statistics was used to assess convergence. The distribution of *Salmonella* counts by level of mesophile contamination were explored by plotting empirical cumulative distribution functions. Chi-square test for trend was used to evaluate *Salmonella* counts across different levels of mesophile contamination.

RESULTS

***Salmonella* outbreaks in Peru (January 2010–January 2021).** A total of 495 foodborne disease outbreaks were reported in Peru between January 2010 and January 2021 (3.7 outbreaks per month), resulting in 14,306 cases of foodborne illness, 2,940 hospitalizations and 51 deaths. These figures are almost certainly a gross underestimation because of underreporting. The likely causative agent was identified in only 66 outbreaks (13.3%), of which 11 were attributed to chemical hazards and the remaining 55 to microbiological hazards. *Salmonella* spp. was the most frequent pathogen, identified as potential causative agent in 31 outbreaks (25 as the only foodborne pathogen involved and 6 together with other pathogens). Outbreaks attributed to *Salmonella* resulted in 1,141 cases, 544 hospitalizations, and three deaths with a median of 30 cases (interquartile range [IQR]: 16–47) and eight hospitalizations (IQR: 1–24) per outbreak.

Information on foods consumed was available for 19 out of the 31 *Salmonella* outbreaks. The most frequently consumed foods were chicken (12 outbreaks), mayonnaise (six outbreaks), and pork (three outbreaks), and only two out of

TABLE 3
Log 10 most probable number per gram (log₁₀ MPN/g) of *Salmonella* found in chicken (*N* = 70) and pork (*N* = 41) samples in six markets from three locations in Peru by individual market, level of contamination, perceived level of hygiene, condition, and cut type

Location	Market	<i>N</i>	Chicken			Pork			
			<i>Salmonella</i>			<i>Salmonella</i>			
			Not Present	0–1.9	≥2	<i>N</i>	Not Present	0–1.9	≥2
Huancayo	A	20	7	13	0	13	8	5	0
	B	10	1	9	0	7	2	5	0
Huaral	C	28	8	16	4	14	13	1	0
	D	2	0	2	0	7	7	0	0
Tumbes	E	6	0	0	6	–	–	–	–
	F	4	0	3	1	–	–	–	–
Level of hygiene									
Acceptable		50	15	31	4	34	28	6	0
Suboptimal		20	1	12	7	7	2	5	0
Condition									
Frozen		7	2	5	0	5	5	0	0
Refrigerated		5	1	3	1	19	15	4	0
Fresh		58	13	35	10	17	10	7	0
Neck cut									
No		34	10	20	4	39	29	10	0
Yes		36	6	23	7	2	1	1	0

Not present indicates the absence of *Salmonella*; 0–1.9 indicates counts ranging from 0 to 1.9 log₁₀ MPN/g and ≥2 indicates counts >2 log₁₀ MPN/g.

the 19 outbreaks, for which food consumed was available, involved foods other than chicken, mayonnaise, or pork.

During the study period, we identified marginal evidence ($P = 0.09$) of spatiotemporal clustering of *Salmonella* outbreaks within a 50-km radius area comprising parts of the Apurimac and Cusco Departments. Within this area, during a 14-week period (October 12, 2018–January 26, 2019), three *Salmonella* outbreaks were identified resulting in 96 cases and 52 hospitalizations (Figure 1).

General appreciations of market hygiene. At market level, observations were done on the type of floor (rough cement or soil versus flattened concrete floor) and ventilation and organization of the stalls. Although there are so many potentially interrelated variables that it is difficult to defend a classification with more than two categories, the markets were categorized into two groups: Markets with “acceptable hygiene” were those with good ventilation, flattened concrete floor (easy to clean), and stalls organized based on the food sold (markets A, C, and D); markets with “suboptimal hygiene” were those with bad ventilation, floor made of either rough cement or soil, and stalls not organized based on type of food (markets B, E, and F).

Overall mesophile quantification and quality acceptability. A total of 70 chicken and 41 pork samples were available for microbiological testing but one chicken sample and two pork samples were missed for mesophiles evaluation. Summary statistics for mesophile counts by species, sampling locations, condition, and type of cut are presented in Tables 2 and 4.

The mean number of mesophiles was 7.23 and 6.91 log₁₀ CFU/g in chicken and pork, respectively. Only one sample from pork in Huaral showed a value lower than 5 log₁₀ CFU/g (4.67 log₁₀ CFU/g) (Table 4), which is acceptable based on the Peruvian normative³² and in this study is in the very low category.

Thirty-one (45%) chicken samples and 20 (51%) pork samples had values between 5 and 7 log₁₀ CFU/g (low category), which could be acceptable or not based on the Peruvian normative. Thirty-eight (55%) chicken samples and 18 (46%) pork samples showed values higher than 7 log₁₀ CFU/g (high category), which is unacceptable according to the Peruvian normative. Regarding the chicken samples by markets and locations, 27.6% (8/29) of samples in Huancayo had a high quantification, including Market A with a 26.3% (5/19) and Market B with a 30.0% (3/10). In Huaral, 70% (21/30) had high quantification values, including Market C with 75% (21/28) and Market D where no samples with a high quantification were found. Finally, in Tumbes, 90% (9/10) had high quantification values, including Market E with a 100% (6/6) and Market F with a 75% (3/4). With respect to the pork samples, in Huancayo, 60% (12/20) of samples had high quantification, including Market A with a 46.2% (6/13) and Market B with 85.7% (6/7); by comparison, in Huaral, 31.6% (6/19) of pork samples had high quantification, including Market C with a 35.7% (5/14) and Market D with a 20% (1/5). In contrast, 60% (12/20) of chicken samples and 85.7% (6/7) of pork samples from markets with an unacceptable level of hygiene had high quantification of mesophiles, whereas a 53.1% (26/49) of chicken samples and a 37.5% (12/32) of pork samples from markets with acceptable hygiene presented such quantification (Table 2).

No evidence of an association was found in the acceptability rating ratios between pork and chicken ($P = 0.34$ by Fisher’s exact test). Differences in mesophile log₁₀ CFU/g were not deemed significant for chicken versus pork ($P = 0.16$ by Wilcoxon test) and between sampling locations for pork ($P = 0.11$ by Wilcoxon test). Differences between locations for chicken were deemed significant ($P = 0.002$ by Kruskal–Wallis test), with the samples from Huaral and Tumbes being more contaminated than those from Huancayo, probably due to, among other causes, the climate of Huancayo, which is in the central Peruvian Andes and has a cooler temperature compared with the other two cities that have higher average temperatures.

Overall *Salmonella* quantitation. A total of 70 chicken and 41 pork samples were evaluated for *Salmonella* contamination. Summary statistics for *Salmonella* results are presented in Table 5, and the sample proportions in different categories of *Salmonella* contamination by market, location, condition, and cut type are presented in Table 3.

All markets had chicken samples positive for *Salmonella*, and all but one market had positive pork samples. *Salmonella* counts were lower for pork than for chicken samples: 95% CI: 0.88–1.34 log₁₀ MPN/g in chicken and 0.10–0.40 log₁₀ MPN/g in pork (Table 5). For the chicken samples, 73.3% (22/30) of samples in Huancayo were positive for *Salmonella*, including Market A with a 65.0% (13/20) and Market B with a 90.0% (9/10). In Huaral 73.3% (22/30) were positive, including Market C with a 71.4% (20/28) and Market D with a 100.0% (2/2). Finally, all chicken samples from Tumbes were positive for *Salmonella*. With respect to the pork samples, 50.0% (10/20) of samples in Huancayo were positive for *Salmonella*, including Market A with a 38.5% (5/13) and Market B with an 71.4% (5/7); by comparison, 4.8% (1/21) of pork samples were positive for *Salmonella* in Huaral, including Market C with a 7.1% (1/14) and Market D with no positive samples. In addition, 95.0% (19/20) of chicken samples

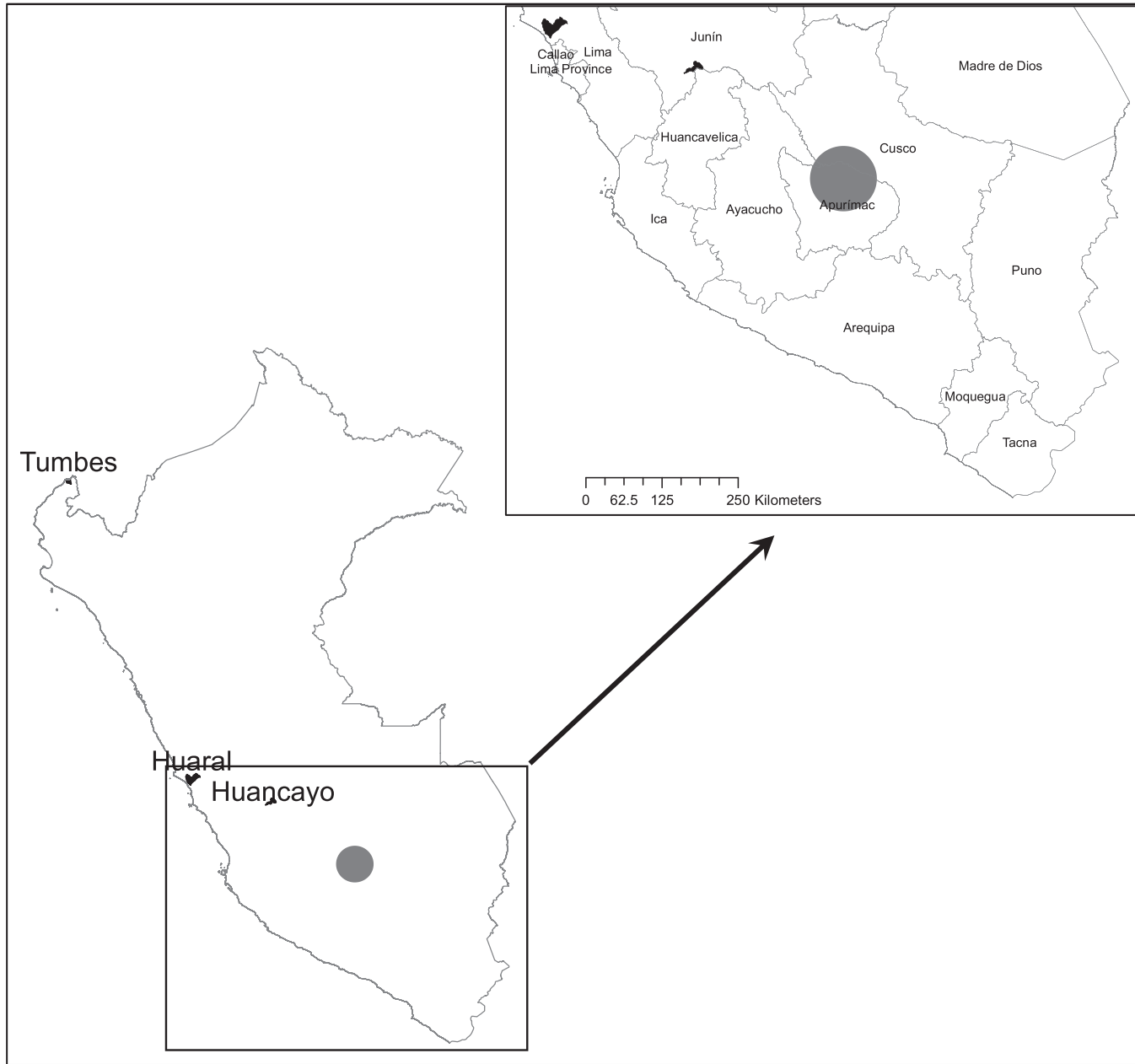


FIGURE 1. Map of Peru displaying the three sampling locations (Huancayo, Huaral, and Tumbes) and a 50-km radius area in which three *Salmonella* outbreaks were observed during a 14-week period providing marginal evidence of spatiotemporal clustering.

TABLE 4

Mean log 10 colony-forming units per gram (log₁₀ CFU/g) of mesophiles found in traditional markets by city in chicken and pork samples, detailing the number of stalls, standard deviation, and 95% CI

Sample by City	Number of Stalls	Mean	SD	95% CI
Chicken				
Huancayo	29	6.83 (a)	0.69	6.57–7.09
Huaral	30	7.42 (b)	0.88	7.09–7.75
Tumbes	10	7.83 (b)	0.78	7.27–8.38
Total	69	7.23	0.86	7.02–7.44
Pork				
Huancayo	20	7.11	0.95	6.67–7.55
Huaral	19	6.69	0.97	6.22–7.16
Total	39	6.91	0.97	6.59–7.22

Letters next to mean values correspond to differences found with a pairwise comparisons using Dunn's all-pairs test.

TABLE 5

Mean log 10 most probable number per gram (log₁₀ MPN/g) of *Salmonella* found in chicken and pork carcasses evaluated in traditional markets from different locations in Peru, detailing the number of stalls, standard deviation, and 95% CI

Sample by City	No. of Stalls	Positive	Mean	SD	95% CI
Chicken					
Huancayo	30	22	0.75 (a)	0.57	0.54–0.97
Huaral	30	22	0.98 (a)	0.89	0.65–1.31
Tumbes	10	10	2.55 (b)	0.78	2.00–3.11
Total	70	54	1.11	0.96	0.88–1.34
Pork					
Huancayo	20	10	0.49	0.58	0.21–0.76
Huaral	21	1	0.02	0.11	–0.03 to 0.07
Total	41	11	0.25	0.47	0.10–0.40

Letters next to mean values correspond to differences found with a pairwise comparisons using Dunn's all-pairs test.

and 71.4% (5/7) of pork samples from markets with a suboptimal level of hygiene were positive for *Salmonella*, whereas *Salmonella* was present in 70.0% (35/50) of chicken samples and a 17.7% (6/34) of pork samples from markets with acceptable hygiene (Table 3).

The log₁₀ MPN/g found by location varied significantly between locations for pork ($P = 0.001$ by Wilcoxon test) and chicken ($P < 0.0001$ by Kruskal–Wallis test), with higher median values found for pork in Huancayo markets than in Huaral. In contrast, chicken samples from Tumbes markets showed significantly higher values compared with Huancayo and Huaral, which had similar values (pairwise comparisons using Dunn's all-pairs test) (Table 5).

Finally, we analyzed the relation between *Salmonella* and mesophiles, and found that in both samples (chicken and pork), high mesophile counts were related with higher quantifications of *Salmonella*. The chicken meat is more likely to have a *Salmonella* quantification higher than 2 log₁₀ MPN/g when it has a high amount of mesophiles ($\chi^2 = 6.82$, $df = 1$, $P = 0.009$), whereas pork meat was more likely to have a quantification between 0 and 1.9 log₁₀ MPN/g when the same amount of mesophiles was encountered ($\chi^2 = 4.35$, $df = 1$, $P = 0.04$) (Table 6).

These findings are shown in Figure 2, which presents the empirical cumulative density function of *Salmonella* count from chicken (Supplemental Figure 1) and pork (Supplemental Figure 2) samples by level of mesophile contamination (low or high). Although the Spearman's correlation between *Salmonella* and mesophiles quantifications was weak (Rho = 0.277, $P = 0.004$), the analysis of the categorical results shows that a meat sample with a high level of mesophiles, an indicator of product contamination, is more likely to present a higher level of *Salmonella* than samples with low levels of mesophiles.

Bayesian beta-binomial model. Finally, a Bayesian approach was used to obtain a better approximation of the confidence intervals of the proportion of contaminated meat in the markets. The estimated probability of *Salmonella* spp. being present in chicken and pork samples is presented in Table 7, stratified by individual market and for samples originating in markets deemed to be of acceptable versus suboptimal hygiene.

The probability of *Salmonella* spp. contamination is consistently high for chicken samples, with lower limit of the credible interval always exceeding a probability of 0.39. The probability of pork contamination is consistently lower than for chicken, although credible intervals in some cases overlap. The probability of infection is higher in markets considered to have suboptimal hygiene practices, with a slight

overlap of credible intervals for chicken samples and no overlap for pork samples (Table 7).

DISCUSSION

Foodborne illness poses a major public health burden worldwide, but lack of data precludes its accurate estimation, particularly in LMICs.¹ Among foodborne pathogens, the WHO has ranked *Salmonella* spp. at the top in terms of global disability adjusted life years (DALYs).¹ In this study, through a retrospective evaluation of the outbreak data and an assessment of the microbiological status of chicken and pork meat on sale in traditional food markets in three locations across Peru, we have obtained possible evidence to support that *Salmonella* spp. present in chicken and pork are a major contribution to foodborne illness in Peru.

Although reporting and diagnosis of foodborne outbreaks in Peru, as in other countries, is incomplete, our findings suggest that *Salmonella* spp. could be the main contributor to the burden of foodborne illness in Peru and that chicken in particular is likely the vehicle of infection. The high proportion of outbreaks for which an etiological agent is not identified and the relatively large number of outbreaks in which *Salmonella* is identified as such, strongly suggest that a many outbreaks, particularly those of relatively small size, are undetected by the system. These multiple factors may have led to the detection of the spatiotemporal clustering of *Salmonella* outbreaks in the area comprising part of both Apurimac and Cusco Departments due to a better outbreak notification system despite being important commercial and touristic areas, respectively. However, given the high percentage of *Salmonella* infection we discovered in chicken samples sold in traditional food markets throughout our investigation (77.1%), eating this contaminated meat may be a major factor in the emergence of these outbreaks, in which *Salmonella* could be the causative agent. It is also important to remember that 70% of Peruvians buy chicken and pork meat in traditional food markets,²⁴ such as those examined in this study. Furthermore, Peru has one of the highest per capita chicken consumption rates in the world, consuming ~4.2 kg/inhabitant/month.²⁴ Although caution is warranted when trying to extrapolate the findings to the country as a whole, the consistency of results across distant locations supports that this is a widespread issue across Peru.

When considering the microbiological results of this study, it is important to emphasize that samples were taken at the end of the food chain, and it was not possible to trace back the actual source of *Salmonella* found in the meat samples. Poultry and pork meat can become contaminated with *Salmonella* at various stages of the production chain, particularly at the slaughterhouse, where carcasses may become contaminated from the environment and/or the intestinal content of infected animals, either from the same or the previously slaughtered batch.^{10,33} Therefore, although *Salmonella* contamination can certainly arise from nonhygienic conditions and practices at the retail stage, markets cannot be solely responsible for the contamination found in this study. However, differences were found in the probability of infection between the two groups of markets based on the level of hygiene. This is an interesting finding, but it should be interpreted with caution because our sampling strategy does not allow us to disentangle the contributions of the

TABLE 6
Log₁₀ most probable number per gram (log₁₀ MPN/g) of *Salmonella* found in chicken and pork samples by amount of mesophiles detected

Sample	Amount of Mesophiles	N	Amount of <i>Salmonella</i>		
			Not Present	0–1.9	≥2
Chicken	High	38	7 (18.4%)	20 (52.6%)	11 (29.0%)
	Low	31	9 (29.0%)	22 (71.0%)	0 (0.0%)
Pork	High	18	10 (55.6%)	8 (44.4%)	0 (0.0%)
	Low	21	18 (85.7%)	3 (14.3%)	0 (0.0%)

Not present indicates the absence of *Salmonella*; 0–1.9 indicates counts ranging from 0 to 1.9 log₁₀ MPN/g and ≥2 indicates counts >2 log₁₀ MPN/g. Low category has values <7 log₁₀ CFU/g and high category >7 log₁₀ CFU/g.

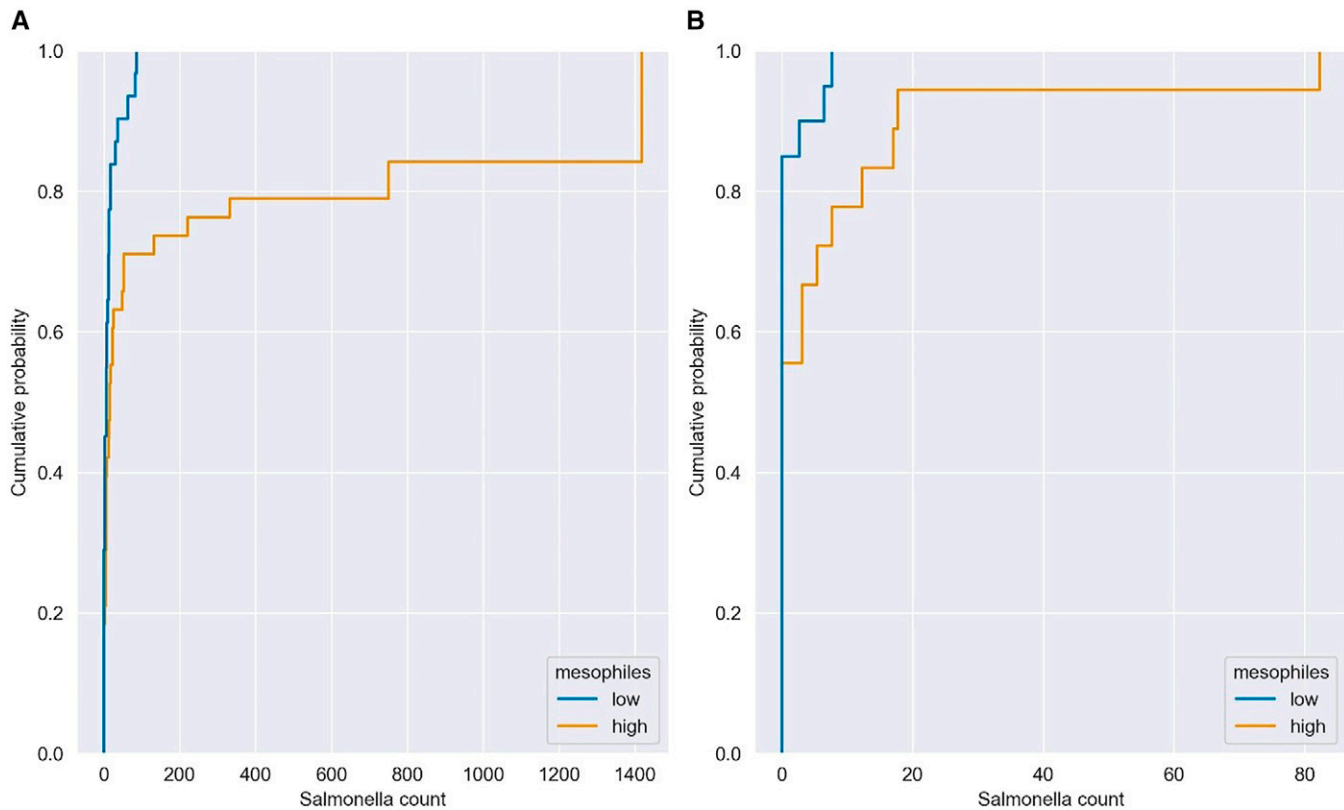


FIGURE 2. Empirical cumulative density function of *Salmonella* count by mesophile count. (A) Samples taken from chicken. (B) Samples taken from pork. The blue line represents the low level of mesophiles, and the orange line the high level of mesophiles.

location and the market itself; further, the classification of markets as having acceptable versus suboptimal hygiene practices is crude and subjective, ignoring within-market variability between stalls.

The implementation of surveillance programs with high coverage to detect *Salmonella* in meat is key to control disease caused by these bacteria. However, the microbiological analysis to detect *Salmonella* is expensive and time-consuming due to the complexity of the organism and the wide variety of strain interventions, in addition to the need to adapt to the specific characteristics of each type of meat product so that the reduction of contamination is effective.³⁴ On the other hand, despite the different laboratory methods

used that make direct comparisons difficult, our results suggest that the levels of *Salmonella* in Peru appears to be much higher than those in other countries, such as the United States.³⁵ All this makes it difficult to implement high-coverage surveillance in terms of budget and logistics, especially in LMICs such Peru, although contamination reduction is possible, as seen in the United States.³⁵ Through the Spearman correlation analysis, a poor correlation ($\rho = 0.28$) was estimated between *Salmonella* and mesophile quantifications. Although this correlation method accepts any type of distribution, a potential attenuation bias produced by a small sample size when estimating variables with high degree of measurement error, such as the microbial concentration, could lead to an underestimation of the correlation coefficient.³⁶ Adjustment methods to reduce the impact of this bias when carrying out correlation analysis have been developed for prevalences using Pearson's method, but these are still lacking for quantification results using non-parametric analysis.³⁷ Despite the weak correlation observed, the analysis of categorized quantifications of both pathogens suggest that meat samples with more than 7 log₁₀ CFU/g of mesophiles may indicate a higher level of *Salmonella* contamination. This means that by applying the cutoff for mesophile quantification set by the Peruvian normative, the majority of *Salmonella*-contaminated pork meat would be thrown out, and more than half of chicken meat would be disposed of, including any samples with quantifications higher than 2 log₁₀ MPN/g. For these reasons, a fast and more economical alternative such the evaluation of

TABLE 7

Estimated probability of *Salmonella* being present in the six markets with 95% credible from a beta-binomial model for chicken and pork samples

Location	Market	Probability of <i>Salmonella</i> (95% CI)	
		Chicken Samples	Pork Samples
Huancayo	A	0.64 (0.45–0.82)	0.40 (0.17–0.62)
	B	0.83 (0.65–0.99)	0.67 (0.38–0.91)
Huaral	C	0.70 (0.55–0.84)	0.13 (0.009–0.28)
	D	0.75 (0.39–1.00)	0.11 (0.00–0.30)
Tumbes	E	0.88 (0.66–1.00)	–
	F	0.83 (0.57–1.00)	–
Level of hygiene			
Acceptable		0.69 (0.58–0.81)	0.20 (0.08–0.31)
Suboptimal		0.91 (0.80–1.00)	0.67 (0.40–0.94)

mesophiles in meat could help partially reduce the presence of meat contaminated with *Salmonella* while these economical and logistical limitations persist.

Our results strongly support prioritization of *Salmonella* control in Peru, particularly in poultry but also in pork. Successful control experiences in other countries show how an integrated approach that considers the entirety of the poultry and pork chains, from farm to fork, is most likely to succeed.³⁸ Such a strategy is also less likely to result in stigmatization of key actors along the food system such as traditional markets, which should be an essential component of a sustainable and equitable food system that delivers safe and nutritious foods to consumers and contributes to local livelihoods. Market vendors will only be able to provide safer chicken and pork meat if *Salmonella* infection is controlled in the farms of origin and contamination is minimized in slaughterhouses.

The high likelihood of *Salmonella* contamination in chicken and pork meat discovered in 2021 in selected markets in three regions of Peru demonstrates the widespread presence of *Salmonella* contamination. This, along with the fact that this bacterium has been linked to the majority of foodborne outbreaks reported in Peru between 2010 and 2021, strongly suggests *Salmonella* as a major cause of foodborne illness in Peru. Chicken and pork are likely to be important vehicles for *Salmonella* infection in Peru, where concerted action tackling *Salmonella* infection along the chicken and pork chains, from primary production to retail sale, is urgently needed.

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