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"*Campylobacter* SPP. EN CARNE DE POLLO DE MERCADOS DE ABASTO Y SU IMPACTO SOBRE LA INCIDENCIA DE CAMPILOBACTERIOSIS HUMANA EN CUATRO REGIONES DE PERÚ: UNA EVALUACIÓN CUANTITATIVA DEL RIESGO MICROBIOLÓGICO"

TESIS PARA OPTAR EL GRADO DE MAESTRA EN CONTROL DE ENFERMEDADES INFECCIOSAS Y TROPICALES

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DEDICATORIA.

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Declaración del Autor

El presente Trabajo de Grado es original y no es el resultado de un trabajo en colaboración con otros, excepto cuando así está citado explícitamente en el texto. No ha sido ni enviado ni sometido a evaluación para la obtención de otro grado o diploma que no sea el presente.

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RESUMEN

Campylobacter es una de las principales causas de gastroenteritis transmitida por alimentos a nivel mundial, siendo la manipulación inadecuada de carne de pollo contaminada una de las principales vías de infección humana. Dada la carga de enfermedad asociada a este patógeno, es necesario realizar evaluar su potencial impacto. Este estudio tuvo como objetivo evaluar la presencia y carga de *Campylobacter* en carne de pollo de mercados de abasto, identificar factores de riesgo relacionados con las condiciones de los puestos de mercado y analizar estrategias de control para la campilobacteriosis en Perú mediante una evaluación cuantitativa del riesgo microbiológico (QMRA). En 2022, se recolectaron y analizaron 90 muestras de carne de pollo de mercados de abasto de Huancayo, Huaral, Tumbes y Piura mediante cultivo bacteriológico y reacción en cadena de la polimerasa en tiempo real cuantitativa (qPCR). *Campylobacter spp*. se detectó en el 28% y 76% de las muestras, con una cuantificación promedio de 3.3 log10 UFC/g y 4.9 log10 GC/g mediante cultivo y qPCR, respectivamente. Los puestos de mercado con acceso a agua corriente mostraron mayor prevalencia y carga, mientras que aquellos sin refrigeradoras presentaron cuantificaciones más altas. El QMRA indicó que toda la población modelada desarrolla campilobacteriosis anualmente, destacando su impacto en la salud pública. Nuestro estudio sugiere que intervenciones a nivel del consumidor, como reducir la contaminación cruzada en la cocina y mejorar el almacenamiento de la carne de pollo, podrían reducir sustancialmente los casos de campilobacteriosis.

PALABRAS CLAVES

CAMPYLOBACTER, CARNE DE POLLO, CULTIVO, qPCR, MERCADOS DE ABASTO, QMRA

ABSTRACT

Campylobacter is a major cause of foodborne gastroenteritis worldwide, with the mishandling of contaminated chicken meat among the main pathways for human infection. Granted the disease burden due to this pathogen, systematic assessments of its potential impact are necessary. This study aimed to evaluate the presence and load of *Campylobacter* in chicken meat from traditional markets, identify risk factors related to market stall conditions, and assess control strategies for campylobacteriosis in Peru using a quantitative microbiological risk assessment (QMRA). Between February and December 2022, a total of 90 chicken meat samples from traditional markets in Huancayo, Huaral, Tumbes and Piura were sampled and evaluated by both culture and quantitative real-time polymerase chain reaction (qPCR). *Campylobacter* spp. were detected in 28% and 76% of samples with a mean quantification of 3.3 $\log_{10}$ CFU/g and 4.9 $\log_{10}$ GC/g through culture and qPCR, respectively. Market stalls with tap water showed higher prevalence and loads, while those without refrigeration had higher quantifications. The QMRA indicated that the entire modeled population develops campylobacteriosis annually. These results highlight the public health impact of *Campylobacter* in the studied populations. Our study suggests that consumer-level interventions, such as reducing kitchen cross-contamination and improving chicken meat storage, could substantially reduce campylobacteriosis cases in this population.

KEYWORDS

CAMPYLOBACTER, CHICKEN MEAT, CULTURE, QPCR, TRADITIONAL MARKETS, QMRA

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Campylobacter spp. in chicken meat from traditional markets in Peru and its impact measured through a quantitative microbiological risk assessment

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ABSTRACT

Campylobacter is a major cause of foodborne gastroenteritis worldwide, with the mishandling of contaminated chicken meat among the main pathways for human infection. Granted the disease burden due to this pathogen, systematic assessments of its potential impact are necessary. The aims of this study were to evaluate both presence and load of *Campylobacter* in chicken meat sold in traditional markets, assess risk factors related with the infrastructure and hygienic conditions of market stalls, and evaluate control strategies for campylobacteriosis in Peru through a quantitative microbiological risk assessment (QMRA), a data-driven, systematic approach to quantitatively assess risks by integrating empirical contamination levels, microbial behavior, and consumer exposure. Between February and December 2022, a total of 90 chicken meat samples from traditional markets were sampled and evaluated by both culture and quantitative real-time polymerase chain reaction (qPCR). *Campylobacter* spp. were detected in 28 % and 76 % of samples with a mean quantification of 3.3 $\log_{10}$ CFU/g and 4.9 $\log_{10}$ GC/g through culture and qPCR, respectively. Market stalls with tap water showed higher prevalence and loads, while those without refrigeration had higher quantifications. The QMRA analysis, using the most conservative parameters and bacterial load, indicated that the entire modeled population develops campylobacteriosis at least once annually. These results highlight the public health impact of *Campylobacter*, potentially linked to the alarming number of Guillain–Barré syndrome cases observed in Peru. Our study suggests that consumer-level interventions, such as reducing kitchen cross-contamination and improving chicken meat storage, could substantially reduce campylobacteriosis cases in this population.

1. Introduction

Campylobacter is the leading cause of human bacterial gastroenteritis worldwide and one of the top four causes of diarrheal disease (Sheppard & Maiden, 2015; WHO, 2020). Diarrheal diseases represent the most frequent foodborne illnesses, with an annual tally of 550 million cases,

Abbreviations: QMRA, Quantitative Microbiological Risk Assessment; sQMRA, swift Quantitative Microbial Risk Assessment Model; qPCR, quantitative real-time polymerase chain reaction; CFU, colony-forming units; GC, genomic copies; DNA, deoxyribonucleic acid; mCCD, modified charcoal cefoperazone deoxycholate; ISO, International Organization for Standardization standards.

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affecting 220 million children under 5 years of age (WHO, 2020). In many countries, *C. jejuni* is responsible for most cases of campylobacteriosis, followed by *C. coli* (FAO/WHO, 2009; Fitzgerald, 2015). Besides causing self-limiting diarrhea, *Campylobacter* infections are associated with serious long-term conditions such as Guillain–Barré syndrome, Miller–Fisher syndrome, and reactive arthritis (Endtz, 2019; Kaakoush et al., 2015). High-risk groups, including young children, the immunocompromised, and the elderly, may experience severe disease, particularly in low- and middle-income countries (WHO, 2020).

Poultry is an important reservoir of *Campylobacter* especially for *C. jejuni*, with 50–80 % of campylobacteriosis cases in the European Union attributed to this host (Koutsoumanis et al., 2020; Skarp et al., 2016). Main transmission pathways are cross-contamination due to mishandling of raw chicken meat contaminated with *Campylobacter* (neck skin being the most contaminated area) and the consumption of undercooked chicken (Ellis-Iversen et al., 2020; Mullner et al., 2009). In Peru, a major consumer of chicken meat, fresh chicken is primarily distributed through traditional markets. However, the role these markets may play in the transmission of *Campylobacter* remains unknown (MIDAGRI, 2022). While current surveillance focuses on prevalence data, incorporating bacterial quantification data is necessary for a more accurate assessment, even though bacterial culture is time-consuming and labor-intensive (Lv et al., 2020). In this regard, the potential use of novel methods such as the quantitative real-time polymerase chain reaction (qPCR) for *Campylobacter* in routine surveillance remains an area of ongoing research (Lv et al., 2020; Magana et al., 2017). Promising results suggest that qPCR could be as effective as traditional culture methods (Stingl et al., 2021).

Quantitative microbiological risk assessment (QMRA) has extensively been used to evaluate public health risk from microbiological foodborne hazards (Chardon & Evers, 2017). It has been used to estimate the impact of the broiler meat chain on human campylobacteriosis in Europe and to inform risk mitigation (Asakura et al., 2022; Koutsoumanis et al., 2020). While comprehensive QMRA of the entire food chain is costly, simplified models using retail data provide a viable alternative for resource-limited countries like Peru (Nauta et al., 2007). This study applies an improved swift QMRA methodology (Chardon & Evers, 2017) to estimate *Campylobacter*'s impact in Peruvian regions with retail data, evaluate control strategies, and provide insights into campylobacteriosis cases obscured by limited surveillance and underreporting.

Given the global impact of this pathogen and the high exposure levels of the Peruvian population (Munayco et al., 2020), surveillance of *Campylobacter* prevalence and concentration in its main food vehicle and the application of this data into risk models like QMRA is an urgent need. For these reasons, this study aimed to i) detect and quantify *Campylobacter* spp. in three Peruvian regions by culture and compare its performance with the faster qPCR method; ii) evaluate selected potential risk factors for *Campylobacter* presence in traditional market stalls; and iii) assess *Campylobacter*'s impact through QMRA, considering bacterial loads find by culture in selected regions of the country, cross-contamination scenarios, and the availability of refrigeration to mitigate bacterial growth and human exposure to the pathogen.

2. Materials and methods

2.1. Sample collection

Samples of fresh raw chicken (n = 90) were randomly collected between February and December 2022 from traditional markets of three Peruvian regions: Huancayo, Huaral, and Tumbes/Piura. Traditional markets were selected based on convenience and feasibility of sample collection. In Huancayo, two markets were sampled; in Huaral, three; and in Tumbes/Piura, four. In each market, stalls were randomly selected, and one sample was collected per selected stall, resulting in a total of 30 samples per region. Samples were transported at 3 – 8 °C in coolers containing gel packs, monitored using the temperature data – logging device TempTale®Ultra (Sensitech Inc., Redmond, WA), and processed microbiologically within 24 h.

At each visit, information on the infrastructure and hygienic conditions of the market stalls was collected through a survey on the Epicollect5 mobile app (Gupta et al., 2021). Field data collectors gathered the following information based on observations of the stalls: acceptability of infrastructure and hygiene, sale of chicken meat only, whether cutting or handling of samples was necessary, degree of cleanliness of the cutting table, degree of cleanliness of the cleaning cloth used on surfaces, and the availability of a refrigerator and tap water in the stall. Further description of this survey is in Supplementary Table S1.

2.2. Identification and quantification by culture technique

Campylobacter spp. detection followed the International Organization for Standardization standards (ISO) 10272–2:2017 (ISO, 2017). Briefly, 10 g of neck-skin from each sample were diluted 1:10 with sterile 1 % buffered peptone water (Merck KGaA, Darmstadt, Germany) and homogenized for two minutes. Immediately, 40 ml of the homogenized solution was stored in a falcon tube at −20 °C for subsequent molecular analysis. Ten-fold serial dilutions were spread-plated onto *Campylobacter* agar with 10 % defibrinated sheep blood and Blaser-Wang supplement (Biomark Laboratories, Pune, India) and in duplicate for quantification onto modified charcoal cefoperazone deoxycholate (mCCD) agar plate with CCDA selective supplement (Merck KGaA, Darmstadt, Germany). Plates were incubated at 41.5 °C for 44 h under microaerophilic conditions using CampyGen™ 2.5L (Oxoid Ltd, Basingstoke, UK). Suspected colonies were Gram-stained and quantified according to ISO 7218:2007(E). For confirmation, colonies were streaked on Columbia agar (Becton, Dickinson and Company, Le Pont de Claix, France) with 5 % defibrinated sheep blood. Standard procedures included Gram staining, motility evaluation, oxidase testing, and assessment of growth under adverse conditions. *Campylobacter jejuni* ATCC 33560 was used as a positive control to validate the procedure.

2.3. Molecular detection and quantification

To evaluate the potential application of qPCR in *Campylobacter* surveillance, we performed a parallel analysis alongside the gold standard culture method on the samples collected. From the initial dilution of each sample stored at −20 °C at the beginning of the culture technique, bacterial DNA extraction was performed according to the recommendations of the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems, Thermo Fisher Scientific, TX, USA). It was carried out from a volume of 200 µL composed by 100 µL of sample and 100 µL of nuclease-free water. In addition, 200 µL of nuclease-free water was used as a negative control to validate the procedure. At the end of the extraction, 50 µL of eluate from each sample was evaluated through a qPCR performed immediately to reduce DNA loss related to storage, freezing and thawing.

The specific qPCR assay described by Josefsen et al. (2004) was used to detect and quantify *Campylobacter* spp. genomic copies (GC). Briefly, a reaction mix was prepared in a final volume of 30 µL using 15 µL of TaqMan® Environmental Master Mix 2.0 (Applied Biosystems, Thermo Fisher Scientific, WA, UK), 0.44 µM of forward primer, 0.48 µM of reverse primer, 0.02 µM of probe and 10 µL of template DNA. All qPCR assays were performed in a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, USA) with the following temperature and time conditions: incubation at 50 °C for 2 min, initial denaturation at 95 °C for 3 min and 45 cycles of denaturation at 95 °C for 15 s, followed by annealing and elongation at 58 °C for 1 min. Automatic settings for threshold and baseline for the determination of positive samples were used. Standard curve was prepared from gBlocks® synthetic DNA fragments (Integrated DNA Technologies, Inc., Coralville, IA, USA) and in a 12-point dilution series (10^9 to 10^{-2} GC). Each sample was analyzed in

sextuplicate (two replicates for the undiluted sample and two replicates for each 10-fold and 100-fold dilution), in order to evaluate the effect of the inhibitory substances. In addition, a standard curve and negative controls was included in each assay. Bacterial DNA extraction, reaction mix preparation, and plate loading were performed in different environments to avoid cross contamination.

2.4. Statistical analysis

The prevalence of positive samples by region and overall across all 3 regions was calculated for results from both culture and qPCR. Differences in prevalence between regions were analyzed using chi-square test and confidence intervals for both techniques. To assess the difference in quantitative results between regions, Kruskal-Wallis and Nemenyi multiple comparison tests were performed using the PMCMRplus package (Pohlert, 2022). Concordance between culture and qPCR techniques was evaluated using McNemar's test and Kappa statistic for qualitative results, while Spearman's correlation was used for the quantitative results.

Furthermore, to assess the association between market stalls infrastructure and hygienic conditions and the proportions of positive samples by culture and qPCR, crude and adjusted prevalence ratios (PR) were estimated with their respective 95 % confidence intervals (95 % CI) through Modified Poisson regression models incorporating robust error estimates (Zou, 2004). The adjusted analysis was made based on the region variable since it was considered a potential confounder. If zero observations are found in any variable, they were randomly replaced with a minimum value (1) for the calculation of the PR. Additionally, the U Mann-Whitney test was used to evaluate the relationship between stalls characteristics and quantitative results from culture and qPCR. To assess the relationship between level of GC obtained by qPCR and the results obtained by culture (the gold standard), a Receiver Operating Characteristic (ROC) curve analysis was performed using the maximum sum of sensitivity and specificity to determine an optimal cut-off point.

Statistical analysis of the collected data in this study was performed using R version 4.2.1 (R-Core-Team, 2015). All the quantifications of colony-forming units per gram (CFU/g) and GC per gram (GC/g) were expressed as the transformation of the logarithm in base 10 ($\log_{10}$).

2.5. Quantitative microbiological risk assessment (QMRA)

The QMRA utilized the enhanced swift Quantitative Microbial Risk Assessment Model (sQMRA), specifically the sQMRA2 version developed by Chardon and Evers (2017) implemented in @Risk (Palisade Corp., NY, USA). This model tracks pathogen levels from retail through consumption, estimating the number of human illness cases. In this study, the chosen sQMRA model was used to simulate the number of cases of human campylobacteriosis in the regions studied and to evaluate possible control strategies. The input parameters needed to run the model, reflecting the situation in Peru, included *Campylobacter* prevalence and quantification results obtained by bacteriological culture of chicken meat purchased at traditional markets following the methodology described in section 2.2. Only culture results were utilized, as the model requires the quantification of viable bacteria to accurately represent the actual risk to the population. After identifying the regions with positive *Campylobacter* culture results, a literature search was conducted on population characteristics and chicken meat consumption, primarily using national surveys and censuses. Moreover, due to the sampled regions having very different climatic conditions, population size, and proportion of inhabitants owning refrigerators (INEI, 2017), the sQMRA was made region-specific. In addition, because of the knowledge gaps in the storage practices at consumer level (i.e. proportion of chicken portions stored at room temperature, in the fridge, and in the freezer by consumers) and the parameters defining the cross-contamination events (i.e. proportion of chicken portions resulting in cross-contamination in consumers' kitchens), different scenarios were run to evaluate the relative impact of these factors.

Scenarios for "cross-contamination" were established to evaluate the risk of *Campylobacter* transmission during chicken preparation in consumer kitchens. These scenarios assumed that 20 %, 40 %, 60 %, and 80 % of chicken portions could potentially cause cross-contamination, where bacteria transfer from the meat to the environment, leading to ingestion through raw salads, kitchen utensils, cutting boards, water, and other surfaces. The factor "storage condition" included three levels where the relative impact of the refrigeration was simulated assuming that 10 % (A), 50 % (B) and 90 % (C) of the people who own refrigerators freeze the chicken. In the model it was assumed that people who do not have a refrigerator store the chicken at room temperature. In addition, several scenarios were simulated in which the current prevalence of contaminated chickens in retail is reduced by 20 %, 40 %, 60 % and 80 %; these scenarios were included to evaluate the impact of hypothetical control options in the number of human cases.

The values characterizing the scenarios for cross-contamination and prevalence were selected to reflect situations of different low to high rates of contamination but without being too extreme in either direction. Values for the storage conditions scenarios were selected to reflect situations where about half (50 %), almost none (10 %) and almost all (90 %) of the people owning a refrigerator would store the chicken in the freezer.

Each simulated scenario consisted of 10,000 iterations and the established outcome for the simulated scenarios were the cases of illness due to *Campylobacter* (campylobacteriosis) per 100,000 inhabitants per year. The pathogen-specific input parameters were taken from the references used by Chardon and Evers (2017) in the "*Campylobacter* on chicken fillet from The Netherlands" model". They include growth and inactivation characteristics of *Campylobacter* based on storage conditions, probability of survival during heating, dose–response parameters and probability of illness given infection.

Ethics clearance was obtained from the Institutional Research Ethics Committee of the Universidad Peruana Cayetano Heredia (Certificate N°516-22-19).

3. Results

3.1. Detection and quantification of Campylobacter spp. in chicken meat from traditional markets

Campylobacter spp. were detected in 27.8 % (25/90) of samples by culture, with an enumeration mean of 0.92 $\log_{10}$ CFU/g (range between 0 and 4.73 $\log_{10}$ CFU/g). On the other hand, qPCR detected a higher proportion of positive samples at 75.6 % (68/90) with an enumeration mean of 3.69 $\log_{10}$ GC/g. These mean quantifications, which include both positive and negative samples, are detailed in Tables 1 and 2. These tables also include mean quantifications of positive samples as they serve as an input parameter in the QMRA model.

Regarding the proportions of positive samples obtained through culture and qPCR, a significant difference was found between Tumbes/Piura and Huancayo and between Tumbes/Piura and Huaral for both techniques (superscripts in the proportion column in Table 1 and 2). Additionally, when analyzing *Campylobacter* concentrations by culture, a significant difference in medians was found between Huaral and Tumbes/Piura, while, by qPCR, the same difference was found but there was also a difference between Huancayo and Huaral.

3.2. Comparison between culture and qPCR results for Campylobacter spp. and ROC curve analysis

The Kappa coefficient for agreement between the qualitative results was 0.22 (95 % CI: 0.12–0.33), reflecting slight agreement between the procedures when interpreted qualitatively. This poor agreement is also reflected in the results of the McNemar test, which provides evidence against the null hypothesis of lack of difference in the proportion of

Table 1
Summary statistics of the results of chicken meat samples from Peruvian traditional markets analyzed by culture for *Campylobacter* spp. The results are shown by region studied and in total.

	Number of samples	Positive	Proportion (95 % CI)	Enumeration mean (log_{10} CFU/g) (Min-Max)	Enumeration mean of positive samples (log_{10} CFU/g) (Min-Max)
Huancayo	30	9	30.0 % [a] (17 % – 48 %)	0.99 [ab] (0 – 4.73)	3.29 (2.80 – 4.73)
Huaral	30	16	53.3 % [a] (36 % – 70 %)	1.77[b] (0 – 4.36)	3.32 (2.14 – 4.36)
Tumbes/ Piura	30	0	0 % [b] (0 % – 11 %)	0 [a]	0
Total	90	25	27.8 % (20 % – 38 %)	0.92 (0 – 4.73)	3.31 (2.14 – 4.73)

95 % CI = 95 % confidence intervals.
Superscripts [a] and [b] next to the proportion of positives correspond to the differences found when comparing the confidence intervals and superscripts [a] and [b] next to the enumeration mean correspond to the differences found using Kruskal Wallis post hoc test.

Table 2
Summary statistics of the results of chicken meat samples from Peruvian traditional markets analyzed by qPCR for *Campylobacter* spp. The results are shown by region studied and in total.

	Number of samples	Positive	Proportion (95 % CI)	Enumeration mean (log_{10} GC/g) (Min-Max)	Enumeration mean of positive samples (log_{10} GC/g) (Min-Max)
Huancayo	30	25	83.33 % [a] (66 % – 93 %)	3.21 [a] (0 – 4.82)	3.85 (3.02 – 4.82)
Huaral	30	30	100 % [a] (89 % – 100 %)	5.80[b] (4.48 – 6.84)	5.80 (4.48 – 6.84)
Tumbes/ Piura	30	13	43.33 % [b] (27 % – 61 %)	2.07 [a] (0 – 5.87)	4.78 (3.61 – 5.87)
Total	90	68	75.56 % (66 % – 83 %)	3.69 (0 – 6.84)	4.89 (3.02 – 6.84)

95 % CI = 95 % confidence intervals.
Superscripts [a] and [b] next to the proportion of positives correspond to the differences found when comparing the confidence intervals and superscripts [a] and [b] next to the enumeration mean correspond to the differences found using Kruskal Wallis post hoc test.

discordant pairs ($p < 0.01$) (Table 3). On the other hand, when the results are treated as numerical, there is a moderate positive correlation between the outcomes of the two methods (Spearman's Rho = 42.4 %; $p < 0.05$).

Regarding the ROC curve analysis, the results show that a cut-off of 5.37 log_{10} GC/g would achieve a sensitivity of 52 % (95 % CI: 0.31–0.72) and a specificity of 85 % (95 % CI: 0.74–0.92) for qPCR (Supplementary Figure S1). Therefore, offering, in settings with the same prevalence that was found in this study, the possibility to detect culture-negative samples with a negative predictive value of 82 % if the output of the qPCR is below that cut-off. Table 3 shows the results obtained considering as a positive sample for qPCR any amplified DNA sample before the automatically configured threshold and baseline whereas Table 4 shows the results that would be obtained by qPCR if the optimal cut-off point of 5.37 log_{10} GC/g was used.

3.3. Factors associated with Campylobacter presence and quantification in chicken meat from traditional markets

Traditional market stall characteristics were analyzed for their association with *Campylobacter* presence, determined by culture and qPCR. In the crude analysis of the culture results, significant variables (p-value < 0.05) included stalls with a refrigerator for meat storage and those with tap water. After adjusting for region, stalls with tap water presented a 3.63-fold increased prevalence of *Campylobacter* compared to those without tap water. Table 5 presents the results of both crude and adjusted analysis using modified Poisson regression. No significant associations were found when the same analysis was performed with the qPCR results.

The relationship between the quantifications of *Campylobacter* obtained by culture and qPCR and the characteristics of traditional market stalls was analyzed using the U Mann-Whitney test. The analysis revealed that the stalls without a refrigerator for meat storage had higher CFU/g and GC/g compared to those with a refrigerator. Additionally, stalls with tap water had higher CFU/g and GC/g compared to those without tap water. The details of the stall characteristics for which the study provided evidence of an association with *Campylobacter* contamination are shown in Table 6.

Table 3
Contingency table of the positive–negative results for *Campylobacter* spp. obtained by culture and qPCR of the chicken meat samples from Peruvian traditional markets in the evaluated regions.

	Culture		Total
qPCR	**Positive**	**Negative**	
Positive	25	43	68
Negative	0	22	22
Total	25	65	

Parameters of qPCR for diagnosis of positive samples (using culture as gold standard): 100% sensitivity, 34% specificity, 37% positive predictive value, and 100% negative predictive value.

Table 4
Contingency table of the positive–negative results applying the cut-off point of 5.37 log_{10} GC/g estimated through ROC curve analysis for qPCR quantifications.

	Culture		Total
qPCR	**Positive**	**Negative**	
Positive	13	10	23
Negative	12	55	67
Total	25	65	

Parameters of qPCR for diagnosis of positive samples (using culture as gold standard): 52% sensitivity, 85% specificity, 57% positive predictive value, and 82% negative predictive value.

Table 5
Crude and adjusted prevalence ratios (PR) of traditional market stall characteristics for the presence of *Campylobacter* spp. by culture calculated using modified Poisson regression analysis.

Variables	Groups		Crude analysis		Adjusted analysis**	
	Positive (n = 25)	Negative (n = 65)	PR (95 % CI)	p-value	PR (95 % CI)	p-value
Acceptable infrastructure and hygiene						
Yes	28.95 % (22)	71.05 % (54)	Ref		Ref	
No	21.43 % (3)	78.57 % (11)	0.74 (0.25 – 2.17)	0.58	1.23 (0.46 – 3.25)	0.68
Stall only sell chicken meat						
Yes	33.33 % (25)	66.67 % (50)	Ref		Ref	
No	0 % (0)*	100 % (15)	0.20 (0.03 – 1.40)	0.10	0.62 (0.94 – 4.09)	0.62
Sample ready to sell (i.e. no cutting or handling required)						
Yes	35.48 % (11)	64.52 % (20)	Ref		Ref	
No	23.73 % (14)	76.27 % (45)	0.67 (0.34 – 1.30)	0.24	0.82 (0.43 – 1.57)	0.55
Degree of cleanliness of the table where the meat is cut						
Clean	12.50 % (2)	87.50 % (14)	Ref		Ref	
Moderately clean	25.81 % (8)	74.19 % (23)	2.07 (0.48 – 8.82)	0.33	2.69 (0.72 – 10.13)	0.14
Dirty	34.88 % (15)	65.12 % (28)	2.79 (0.70 – 11.12)	0.15	2.41 (0.68 – 8.53)	0.17
Degree of cleanliness of the cleaning cloth used on the surfaces						
Clean	12.50 % (2)	87.50 % (14)	Ref		Ref	
Moderately clean	27.27 % (12)	72.73 % (32)	2.18 (0.53 – 8.91)	0.28	2.69 (0.73 – 10.00)	0.14
Dirty	36.67 % (11)	63.33 % (19)	2.93 (0.72 – 11.93)	0.13	3.09 (0.84 – 11.36)	0.09
Stall with a refrigerator to store meat						
Yes	22.54 % (16)	77.46 % (55)	Ref		Ref	
No	47.37 % (9)	52.63 % (10)	2.10 (1.10 – 4.03)	0.025	1.77 (0.96 – 3.27)	0.07
Stall with tap water						
No	18.18 % (8)	81.82 % (36)	Ref		Ref	
Yes	36.96 % (17)	63.04 % (29)	2.03 (0.97 – 4.26)	0.06	3.63 (1.98 – 6.63)	<0.001

* The value 0 was randomly changed to 1 to calculate the PR presented.
** Adjusted by region using modified Poisson regression analysis.

Table 6
Analysis of traditional market stall characteristics and quantitative *Campylobacter* results by culture and qPCR using the U Mann-Whitney test, showing only variables with strong evidence of association.

Variables	Culture Mean (Log_{10} CFU/g)	U Mann-Whitney test p-value	qPCR Mean (Log_{10} GC/g)	U Mann-Whitney test p-value
Stall with a refrigerator to store meat				
Yes	0.77	0.05	3.47	0.032
No	1.49		4.52	
Stall with tap water				
Yes	1.24	0.029	4.48	< 0.001
No	0.59		2.87	

3.4. Simulation of human campylobacteriosis cases and control strategies assessment using QMRA modeling

To carry out this analysis, both *Campylobacter* prevalence and quantifications obtained by culture in this study were used. Since the Tumbes/Piura region did not have positive results by culture, only scenarios for Huaral and Huancayo regions were simulated. After identifying the regions with positive results, the input parameters specific to the QMRA scenarios for each region were developed based on both our study's findings and a literature review. These parameters are detailed in Tables 7, 8 and 9.

Using the input parameters described above, we employed the sQMRA model to simulate annual campylobacteriosis cases per 100,000 inhabitants in each region. The simulations considered different scenarios, including chicken portion storage conditions, the percentage of portions that could cause cross-contamination at the consumer level, and the reduction of the prevalence identified in this study of *Campylobacter*-contaminated chicken sold in traditional markets. This approach allowed us to quantify the impact of these parameters on the annual incidence rate of campylobacteriosis in each region. As a result, in the most conservative scenario, the annual number of campylobacteriosis cases was around 104,405 per 100,000 habitants.

Additionally, through simulated scenarios, we evaluated how control strategies focusing on improving storage conditions, reducing cross-contamination, and lowering the prevalence of positive samples could potentially reduce case numbers. Regarding the simulated scenarios for storage conditions, the median number of campylobacteriosis cases decreases as more people store chicken meat in the freezer. When the percentage of refrigerator owners who store chicken at freezing temperatures increases from 10 % to 90 %, campylobacteriosis cases decrease by 40 % in Huaral and by 22 % in Huancayo, approximately. Additionally, when the percentage of chicken servings likely to cause cross-contamination at the consumer level is reduced, the decrease in median campylobacteriosis cases is similar across all three storage condition scenarios in both regions. Cases approximately decrease by 25 % when cross-contamination is reduced from 80 % to 60 %, by 50 % when reduced from 80 % to 40 %, and by 75 % when reduced from 80 %

Table 7
Input parameter values with reference/source used in the QMRA model.

Scope			
Pathogen	*Campylobacter*		
Food product	Chicken meat		
Population specification	Tables 8 and 9		
Population size	Tables 8 and 9		
Consumption period in days	365		
Question	**Value**	**Unit**	**Ref**
1 Portions consumed			
Point estimation: portions per person per month (pppm)	30.42	/pppm	(MIDAGRI, 2022)
2 Pathogen prevalence in retail			
Point estimation of prevalence	Table 9	–	–
3. Portion size			
Portion size: mean	139.55	g	(MIDAGRI, 2022)
Portion size: st dev	6.71	g	
4 Pathogen concentration			
Concentration: mean	Table 9	log_{10} (CFU/g)	–
Concentration: st dev	Table 9	log_{10} (CFU/g)	
5 Storage conditions			
% of portions stored in room	Tables 8 and 9	–	–
% of portions stored in the fridge	Tables 8 and 9	–	–
% of portions stored in freezer	Tables 8 and 9	–	–
7 Cross-contamination parameters			
% of portions possibly causing cross-contamination	Tables 8 and 9	–	–
8 Heating categories			
% of portions done	95	%	Authors' estimate
% of portions undercooked	5	%	
% of portions raw	0	%	

Table 8
Storage conditions and cross-contamination scenarios simulated in the QMRA model.

Storage conditions scenarios		Region Huaral	Huancayo	Ref
A	% of portions stored in the fridge	56.93 %	32.66 %	(INEI, 2017)
	% of portions stored in freezer	6.33 %	3.63 %	
B	% of portions stored in the fridge	31.63 %	18.15 %	
	% of portions stored in freezer	31.63 %	18.15 %	
C	% of portions stored in the fridge	6.33 %	3.63 %	
	% of portions stored in freezer	56.93 %	32.66 %	
A, B, C	% of portions stored at room temperature	36.75 %	63.71 %	

A, B and C are, respectively, scenarios where 10%, 50% and 90% of the people have a refrigerator in which they keep their chicken portions in the freezer. For each scenario of storage conditions, four cross-contamination scenarios were simulated (20%, 40%, 60% and 80%).

to 20 % (Fig. 1).

Finally, in the simulated scenarios where the current prevalence of contaminated chicken meat at traditional markets is decreased, the reduction in median campylobacteriosis cases closely matched the percentage decrease in the prevalence of contaminated chicken meat for both regions. Specifically, when the current prevalence is reduced by 20 %, 40 %, 60 %, and 80 %, the median cases decrease by approximately 20 %, 41 %, 61 %, and 81 %, respectively (Fig. 2).

4. Discussion

In this study, *Campylobacter* spp. were detected and quantified in chicken meat from traditional markets in Peru and QMRA was used to assess the risk of consuming contaminated chicken in the evaluated regions. *Campylobacter* was studied since it is a major cause of gastroenteritis globally, and because despite being associated with an outbreak of Guillain-Barre syndrome in Peru in 2019, it remains poorly understood in the country (Munayco et al., 2020; CDC-MINSA, 2023). Furthermore, reliable quantitative data on viable pathogenic bacteria are necessary for assessing food-related health risks and understanding bacterial interactions to inform decision-making (Newell & Fearnley, 2003). Although Peruvian regulations require an absence of *Campylobacter* in chicken sold for consumption (MINSA-PERU, 2008), no efforts have been made yet for the situational diagnosis or quantification of this microorganism. In contrast, the European Union sets a limit of up to 1000 CFU/g in broiler carcasses, which has been shown to reduce human campylobacteriosis incidence by two-thirds (Swart et al., 2013). Given that eradicating this pathogen at the farm level is not yet possible in European countries (Georgiev et al., 2017), a similar situation could be expected in Peru.

4.1. *Campylobacter spp.* in chicken meat from traditional markets

This study found a mean *Campylobacter* concentration of 3.31 $\log_{10}$ CFU/g in 27.8 % of positive samples through the culture technique. These results align with concentrations reported globally, including 3 $\log_{10}$ CFU/g in Eastern Europe (Mäesaar et al., 2014), 2.7 $\log_{10}$ in United Arab Emirates from 28.6 % of contaminated samples (Habib et al., 2022) and 3.1 $\log_{10}$ CFU/g in Brazil (Pozza et al., 2020). On the other hand, we found higher concentrations than in Australia which reported 1.8 $\log_{10}$ CFU/g (Habib et al., 2018) and Lithuania which reported concentrations between 1.99 and 2.11 $\log_{10}$ CFU/g (Bunevičienė et al., 2010). However, there are limited studies worldwide on quantifying *Campylobacter* in chicken meat using culture techniques, and to our knowledge, this is the first study of its kind in Peru.

Campylobacter concentration in chicken meat depends on initial flock contamination, handling during transport, slaughter process, time between slaughter and sale, and storage methods until consumption (Newell et al., 2017). Although our analysis focused on traditional markets, it is important to consider the time the chicken is being transported since crates are an important factor in their exposure to pathogens and subsequent colonization. For example, chicken consumed in Huancayo are transported alive for approximately 10–12 h to be immediately slaughtered, this could increase stress in chickens which might reduce their immunocompetence making them more susceptible to *Campylobacter*. Research indicates that transport stress

Table 9
Prevalence scenarios simulated in the QMRA model.

Input parameters	Prevalence scenarios Current prevalence	20 % reduction	40 % reduction	60 % reduction	80 % reduction	Ref
Region of Huaral						
Population size	183,898					(INEI, 2017)
Pathogen prevalence in retail						
Point estimation of prevalence	53.3 %	42.6 %	32 %	21.3 %	10.7 %	Results of this study
Pathogen concentration						
Concentration: mean	3.32 $\log_{10}$ (CFU/g)					Results of this study
Concentration: st dev	0.58 $\log_{10}$ (CFU/g)					
Storage conditions						
% of portions stored at room temperature	36.75 % for all scenarios					(MIDAGRI, 2022)
% of portions stored in the fridge	56.93 % for all scenarios					
% of portions stored in the freezer	6.33 % for all scenarios					
Cross-contamination parameters						
% of portions possibly causing cross-contamination	10 % for all scenarios					Authors' estimate
Region of Huancayo						
Population size	545,615					(INEI, 2017)
Pathogen prevalence in retail						
Point estimation of prevalence	30 %	24 %	18 %	12 %	6 %	Results of this study
Pathogen concentration						
Concentration: mean	3.29 $\log_{10}$ (CFU/g)					Results of this study
Concentration: st dev	0.59 $\log_{10}$ (CFU/g)					
Storage conditions						
% of portions stored at room temperature	63.71 % for all scenarios					(MIDAGRI, 2022)
% of portions stored in the fridge	32.66 % for all scenarios					
% of portions stored in the freezer	3.63 % for all scenarios					
Cross-contamination parameters						
% of portions possibly causing cross-contamination	10 % for all scenarios					Authors' estimate

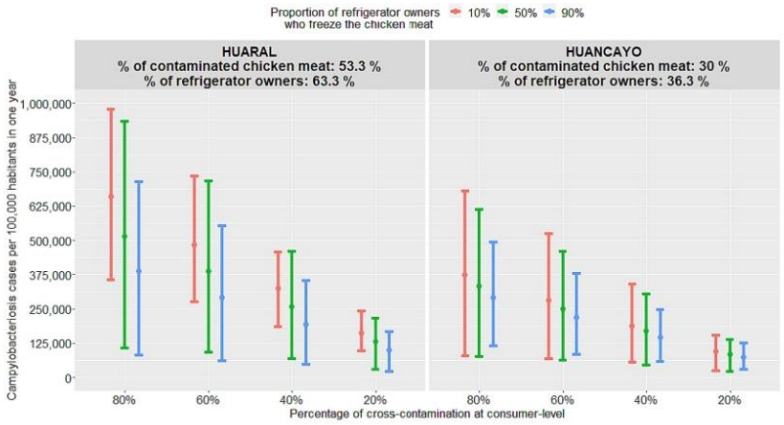

Fig. 1. Simulated cross-contamination scenarios with different storage conditions. The X-axis represents cross-contamination scenarios at the consumer level, with each color representing different percentages of people storing chicken meat at freezing temperatures. Points indicate the median incidence rate of campylobacteriosis, and error bars represent the 90% credible intervals for each scenario.

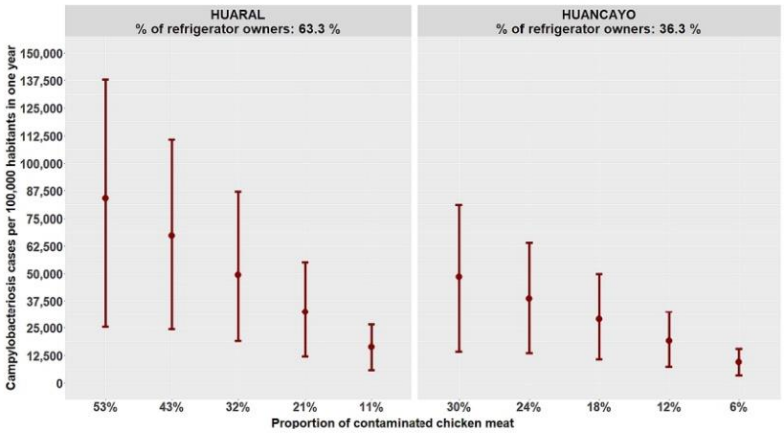

Fig. 2. Simulated scenarios for the percentage reduction of the current prevalence of contaminated chicken meat for Huaral and Huancayo. The X-axis represents current prevalence levels in markets and their reduction by 20%, 40%, 60%, and 80%. Points indicate the median incidence rate of campylobacteriosis, and error bars show the 90% credible intervals for each scenario.

increases *Campylobacter* excretion rates, with colonization occurring within 4 h in contaminated crates (Rasschaert et al., 2020). This was not found in poultry transported for only 2 h (Slader et al., 2002). In contrast to Huancayo, chicken from Huaral experience shorter transport times, suggesting other factors may influence *Campylobacter* concentrations found. Therefore, transport crates may play an important role in cross-contamination of chicken carcasses in the markets from Huancayo, but less so in the markets from Huaral.

Campylobacter was not detected in Tumbes/Piura through culture technique. However, the genetic material found through qPCR demonstrates the possibility of the presence of dead, non-viable bacteria, or concentrations below the detection limit of the culture method. This region has an average annual temperature of 24.2 °C (INEI, 2022b), so farms usually have more ventilation control, and most traditional market stalls have refrigerators, which may reduce bacterial concentrations in the analyzed samples. On the other hand, the viability of *Campylobacter* could have been affected at some point between the slaughterhouse and the retail sale. Fresh chicken sold may have been frozen without consumer's knowledge, and this could have led to *Campylobacter* entering a viable but non-culturable state, which has been described under stress conditions such as low temperatures and aerobic environments, making it undetectable by culture techniques (Lv et al., 2020). It would be important to continue monitoring the presence and load of viable *Campylobacter* in chicken meat, as Tumbes/Piura is a region where an outbreak of Guillain-Barre Syndrome was reported shortly after our evaluation (CDC-MINSA, 2023).

qPCR detected more positive samples than culture, but the results should be interpreted with caution since they may not reflect actual

population risk. This is because the qPCR assay used in this study detects both viable and non-viable cells, potentially inflating prevalence estimates (Josefsen et al., 2004; Magana et al., 2017). Although promising qPCR assays capable of distinguishing alive from dead cells are under development, they have not yet replaced the gold standard of bacteriological culture (Dubovitskaya et al., 2023; Thornval & Hoorfar, 2021). Thus, qPCR was used in this study to complement culture and to evaluate its potential as a tool for routine *Campylobacter* surveillance in chicken meat, as discussed in Section 4.3.

4.2. Role of traditional market stall characteristics in Campylobacter spp. prevalence and quantification

Regarding the traditional market stall characteristics, access to tap water was associated with higher *Campylobacter* prevalence and higher quantifications by both culture and qPCR analysis. Studies have demonstrated that this pathogen can rapidly spread on food and nearby surfaces (Habib et al., 2020; Sarjit & Dykes, 2017), suggesting that tap water in the stall might represent a risk factor by facilitating the spread of bacteria when washing utensils used on contaminated carcasses, such as chopping boards, knives, and cleaning cloths. In fact, campaigns have highlighted the importance of not washing chicken at home to reduce cross-contamination risks (Henley et al., 2016). Moreover, sellers could be washing the chicken meat to remove organic residues, making it more appealing to consumers. On the other hand, not having a refrigerator for chicken storage was related to higher *Campylobacter* quantifications by culture and qPCR, despite not being associated with a higher prevalence. This could be attributed to refrigeration reducing bacterial concentrations by up to 0.81 $\log_{10}$ CFU per gram (Bhaduri & Cottrell, 2004), while freezing meat for a few days decreases *Campylobacter* concentration by up to 1.4 $\log_{10}$ CFU/g and if it is done for 28–31 days decreases it by up to 2.33–2.9 $\log_{10}$ CFU/g (Georgsson et al., 2006; Maziero & de Oliveira, 2010). In this manner, traditional market stall characteristics might be playing an important role in *Campylobacter* transmission to consumers through contaminated chicken meat.

4.3. Potential use of qPCR in Campylobacter surveillance

Surveillance of *Campylobacter* spp. in many countries is hindered by the complex methodology required for isolation and quantification via culture, which demands immediate processing after sampling and can take up to seven days (Magana et al., 2017). Conversely, conventional molecular techniques enable detection within a day but only identify genetic material, leaving uncertainty regarding the pathogen's viability in the meat and its potential risk for consumers. Our evaluation, using ROC curve analysis, suggests a cut-off point of 5.37 $\log_{10}$ GC/g to determine if a sample would be negative by culture with a specificity of 85 % (95 % CI: 0.74–0.92). This could allow a rapid assessment of chicken meat batches using molecular techniques, ensuring they meet safety standards before reaching retail markets, while culture analysis is conducted concurrently. However, the proposed cut-off point requires validation before being implemented as a complementary diagnostic tool in *Campylobacter* surveillance.

4.4. Simulated cases of human campylobacteriosis through sQMRA based on prevalence and quantification data from traditional markets

In our QMRA simulations we obtained risks that were 600 times higher those reported in the baseline "*Campylobacter* on chicken fillet from The Netherlands" model (Chardon & Evers, 2017). In Peru, consumption, storage conditions, and cross-contamination levels for chicken differ from those in the Netherlands. A hypothetical scenario using only the prevalence and concentration of *Campylobacter* from this study but maintaining Dutch parameters shows a risk 35 times higher, highlighting the impact of bacterial load. However, the remaining impact of this disease may also stem from chicken being Peru's primary protein source, with per capita consumption 15 times higher than in the Netherlands (MIDAGRI, 2022). In Peru as in other low- and middle-income countries, washing raw chicken to remove visible residues is common, as many believe it makes it safer (Joao et al., 2021). However, this practice can spread bacteria to other kitchen surfaces (Sarjit & Dykes, 2017), especially if the same cutting board is used for raw chicken and vegetables, increasing transmission risk. This reflects the paramount importance of maintaining good kitchen hygiene practices to avoid cross-contamination during handling of *Campylobacter*-contaminated chickens to reduce the risk of campylobacteriosis (Eriksson et al., 2023).

This analysis indicates that, using conservative parameters and the bacterial load identified by culture in our study, every consumer in the evaluated regions would develop campylobacteriosis at least once annually. This alarming finding highlights the potential severity of the problem. On the other hand, limitations in Peru's syndromic surveillance of acute gastroenteritis and foodborne illnesses lead to underreporting of campylobacteriosis cases, making it difficult to fully rule out annual incidence rates in the simulated scenarios. Consequently, the QMRA section in this study focuses on discussing mainly the control strategies evaluated in the analysis rather than the simulated incidence rates. Nevertheless, it is important to emphasize the need to characterize the agent in both acute gastroenteritis outbreaks and routine surveillance.

4.5. Control strategies for human campylobacteriosis evaluated through QMRA simulations

Among the campylobacteriosis control strategies evaluated through QMRA simulations, reducing cross-contamination at the consumer level and increasing the fraction of the population with refrigerators that store chicken at freezing temperatures are realistic options. These strategies could be implemented through educational campaigns without requiring investment in equipment. This study assumes that the percentage of chicken portions causing cross-contamination in Peru ranges from 40 – 80 %. Despite this high figure, we demonstrated that reducing cross-contamination from 80 % to 20 % could decrease the median annual incidence rate of campylobacteriosis by up to 75 %. This is consistent with previous studies indicating that effective control of cross-contamination markedly reduces the risk of *Campylobacter* transmission (Cardoso et al., 2021; Lindqvist & Lindblad, 2008). On the other hand, while having a refrigerator improves quality of life (Karlsson & Subramanian, 2023), only 55.1 % of Peruvians currently own one, and expanding this coverage would require substantial economic investment (INEI, 2022a). Nevertheless, increasing the proportion of people who store chicken in the freezer from 10 % to 90 % while maintaining the current number of refrigerator owners could decrease campylobacteriosis cases by nearly 40 %, likely due to the significant reduction in pathogen concentration after freezing (Georgsson et al., 2006). This finding aligns with the studies that demonstrate that cold and freezing temperatures effectively reduce *Campylobacter* loads, thus lowering the risk of infection (Nastasijevic et al., 2020; Thames & Sukumaran, 2020). Therefore, it is necessary that corresponding authorities implement education programs to promote proper raw chicken meat handling practices, raise awareness about the role of cross-contamination in the transmission of campylobacteriosis, and encourage the storage of chicken in the freezer to reduce the incidence rate.

The percentage reduction in simulated cases relative to the decrease in the prevalence of contaminated chicken on traditional markets highlights the importance of efforts to reduce the level of contamination in primary production in order to provide safer meat to consumers. Nevertheless, even with an increase of biosecurity at the farm level to extremely high standards, a high proportion of poultry batches would still be contaminated (Georgiev et al., 2017). This issue lacks a single solution; instead, there are multiple vulnerable points in the production chain where the different stakeholders, including the authorities, can

intervene. Therefore, improvements must be developed at the level of the different critical points throughout the production chain to mitigate the public health impact of this pathogen.

Our study had limitations, including the potential selection bias related to the traditional markets sampled, which could overestimate the prevalence of *Campylobacter*. To minimize it, we selected markets with the highest attendance in each region. Furthermore, this study did not differentiate between *Campylobacter* species in the chicken samples, and the sQMRA model used was parameterized with *C. jejuni* dose–response data. As species distribution in the evaluated regions is unknown, extrapolating disease impact to the Peruvian context without specific epidemiological data introduces a potential bias that could overestimate or underestimate the results of the presented QMRA.

It is also important to acknowledge that the QMRA outputs rely on a number of simplifications and assumptions and require further validation in real-world contexts. Despite this, these results offer a valuable foundation for understanding campylobacteriosis control dynamics. The reliance on input parameters that may not fully reflect the diverse consumer practices regarding cross-contamination and storage could affect the applicability of the results. Variations in consumer behavior across different regions may lead to over- or underestimations of the effectiveness of the proposed interventions. Consequently, current information on cross-contamination levels and chicken meat storage practices at the consumer level in Peru is needed for more accurate estimations of campylobacteriosis cases.

Finally, the QMRA model used in this study does not consider immunity due to previous *Campylobacter* infections potentially leading to an overestimation of simulated cases. While recurrent infections can occur in adults, exposure to this pathogen at an early age in endemic settings may induce protective immunity (Kaakoush et al., 2015). Therefore, future studies should focus on the efficiency and duration of immunity produced by *Campylobacter* to enhance available QMRA models.

5. Conclusions

In conclusion, *Campylobacter* is present in chicken meat sold in traditional markets in the three regions evaluated, at least by molecular analysis. Culture results indicated a high mean concentration in CFU/g, which does not comply with any of the sanitary regulations for the sale of chicken meat. Additionally, our findings indicate that improper use of tap water and inadequate refrigeration practices in market stalls are likely contributors to the observed *Campylobacter* contamination. This represents an important risk to public health, which was corroborated with the development of QMRA models that demonstrated that cross-contamination and storage conditions at the consumer level play an important role in the transmission dynamics of human campylobacteriosis. In light of the high levels of contamination reported in this study and the control strategies assessed in the QMRA, it is necessary to enhance food hygiene practices along the poultry production chain and among consumers to mitigate the risk of acquiring campylobacteriosis. While the outputs of our QMRA model need further validation, they represent a valuable starting point for understanding these dynamics. For these reasons, the findings of this study could contribute to the establishment of prevention and control measures by the corresponding authorities.

6. Author disclaimer

Matteo Crotta is employed by the European Food Safety Authority (EFSA). However, the present article is published under the sole responsibility of the authors and may not be considered as an EFSA scientific output. The positions and opinions presented in this article are those of the authors alone and do not necessarily represent the views/any official position or scientific works of EFSA. To know about the views or scientific outputs of EFSA, please consult its website "https://www.efsa.europa.eu".

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115424.

Data availability

Data will be made available on request.

References

Asakura, H., Yamamoto, S., Yamada, K., Kawase, J., Nakamura, H., Abe, K., Sasaki, Y., Ikeda, T., & Nomoto, R. (2022). Quantitative detection and genetic characterization of thermotolerant *Campylobacter* spp. in fresh chicken meats at retail in Japan. *Frontiers in Microbiology, 13*(11). https://doi.org/10.3389/fmicb.2022.1014212

Bhaduri, S., & Cottrell, B. (2004). Survival of cold-stressed *Campylobacter jejuni* on ground chicken and chicken skin during frozen storage. *Applied and Environmental Microbiology, 70*(12), 7103–7109. https://doi.org/10.1128/AEM.70.12.7103-7109.2004

Bunevičienė, J., Kudirkienė, E., Ramonaite, S., & Malakauskas, M. (2010). Occurrence and numbers of *Campylobacter* spp. on wings and drumsticks of broiler chickens at the retail level in Lithuania. *Veterinarija ir zootechnika, 50*(72), 9–14.

Cardoso, M. J., Ferreira, V., Truninger, M., Maia, R., & Teixeira, P. (2021). Cross-contamination events of *Campylobacter* spp. in domestic kitchens associated with consumer handling practices of raw poultry. *International Journal of Food Microbiology, 338*. https://doi.org/10.1016/j.ijfoodmicro.2020.108984

CDC MINSA. (2023). Alerta epidemiologica: Incremento de casos de síndrome de Guillain-Barré en algunas regiones del país. Retrieved from https://www.dge.gob.pe/epipublic/uploads/alertas/alertas_202315_26_141558.pdf. Accessed March 9, 2024.

Chardon, J. E., & Evers, E. G. (2017). Improved swift Quantitative Microbiological Risk Assessment (sQMRA) methodology. *Food Control, 73*, 1285–1297. https://doi.org/10.1016/j.foodcont.2016.10.049

Dubovitskaya, O., Seinige, D., Valero, A., Reich, F., & Kehrenberg, C. (2023). Quantitative assessment of *Campylobacter* spp. levels with real-time PCR methods at different stages of the broiler food chain. *Food Microbiology, 110*. https://doi.org/10.1016/j.fm.2022.104152

Ellis-Iversen, J., Gantzhorn, M. R., Borck Høg, B., Foddai, A., & Nauta, M. (2020). The ability to detect *Campylobacter* presence and concentration using different chicken carcass samples. *Food Control, 115*, Article 107294. https://doi.org/10.1016/j.foodcont.2020.107294

Endtz, H. P. (2019). *Campylobacter* Infections. In E. T. Ryan, D. R. Hill, T. Solomon, N. E. Aronson, & T. P. Endy (Eds.), Hunter's Tropical Medicine and Emerging Infectious Disease (10th ed, pp. 507–511). Elsevier Inc. https://doi.org/10.1016/B978-0-323-55512-8.00050-8.

Eriksson, D., Råhlén, E., Bergenkvist, E., Skarin, M., Fernström, L. L., Rydén, J., & Hansson, I. (2023). Survival of *Campylobacter jejuni* in frozen chicken meat and risks associated with handling contaminated chicken in the kitchen. *Food Control, 145*. https://doi.org/10.1016/j.foodcont.2022.109471

[FAO/WHO] Food and Agriculture Organization of the United Nations/World Health, & Organization. (2009). Risk assessment of *Campylobacter* spp. in broiler chickens: Technical report. In Microbiological Risk Assessment Series N° 12.

Fitzgerald, C. (2015). Campylobacter. *Clinics in Laboratory Medicine, 35*, 289–298. https://doi.org/10.1016/j.cll.2015.03.001

Georgiev, M., Beauvais, W., & Guitian, J. (2017). Effect of enhanced biosecurity and selected on-farm factors on *Campylobacter* colonization of chicken broilers. *Epidemiology and Infection, 145*(3), 553–567. https://doi.org/10.1017/S095026881600251X

Georgsson, F., Þorkelsson, Á. E., Geirsdóttir, M., Reiersen, J., & Stern, N. J. (2006). The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. *Food Microbiology, 23*(7), 677–683. https://doi.org/10.1016/j.fm.2005.10.003

Gupta, S., Sharma, S., Gohil, R., & Sachdeva, S. (2021). Epicollect5: A free, fully customizable mobile-based application for data collection in clinical research. *Journal of Postgraduate Medicine, Education and Research, 54*(4), 248–251. https://doi.org/10.5005/jp-journals-10028-1375

Habib, I., Coles, J., Fallows, M., & Goodchild, S. (2018). A baseline quantitative survey of *Campylobacter* spp. on retail chicken portions and carcasses in Metropolitan Perth, Western Australia. *Foodborne Pathogens and Disease, 16*(3), 180–186. https://doi.org/10.1089/fpd.2018.2554

Habib, I., Harb, A., Hansson, I., Vågsholm, I., Osama, W., Adnan, S., Anwar, M., Agamy, N., & Boqvist, S. (2020). Challenges and opportunities towards the development of risk assessment at the consumer phase in developing countries—The case of *Campylobacter* cross-contamination during handling of raw chicken in two middle eastern countries. *Pathogens, 9*(1), 62. https://doi.org/10.3390/pathogens9010062

Habib, I., Yousif, M., Mohamed, I., Lakshmi, G. B., Khan, M., & Li, D. (2022). Quantification of *Campylobacter* contamination on chicken carcasses sold in retail markets in the United Arab Emirates. *International Journal of Food Contamination, 9*(9), 1–8. https://doi.org/10.1186/s40550-022-00095-4

Henley, S. C., Gleason, J., & Quinlan, J. J. (2016). Don't wash your chicken!: A food safety education campaign to address a common food mishandling practice. *Food Protection Trends, 36*(1), 43–53.

[INEI] Instituto Nacional de Estadística e Informática. (2017). Censos Nacionales 2017: XII de Población, VII de Vivienda y III de Comunidades Indígenas. Retrieved from https://censo2017.inei.gob.pe/resultados-definitivos-de-los-censos-nacionales-2017/. Accessed November 11, 2023.

[INEI] Instituto Nacional de Estadística e Informática. (2022a). Encuesta nacional de hogares: Condiciones de vida en el Perú. Informe técnico N° 02 junio. Retrieved from https://m.inei.gob.pe/media/MenuRecursivo/boletines/informe_de_condiciones_de_vida_ene-feb_mar2022.pdf. Accessed December 13, 2023.

[INEI] Instituto Nacional de Estadística e Informática. (2022b). Perú: Anuario de Estadísticas Ambientales 2022. Retrieved from https://www.inei.gob.pe/media/MenuRecursivo/publicaciones_digitales/Est/Lib1877/libro.pdf. Accessed December 15, 2023.

ISO. (2017). International Organization for Standardization 10272-2: 2017. Microbiology of the food chain - horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique. 1, 19.

Joao, M., Ferreira, V., Truninger, M., Maia, R., & Teixeira, P. (2021). Cross-contamination events of *Campylobacter* spp. in domestic kitchens associated with consumer handling practices of raw poultry. *International Journal of Food Microbiology, 338*. https://doi.org/10.1016/j.ijfoodmicro.2020.108984

Josefsen, M. H., Cook, N., Agostino, M. D., Hansen, F., Wagner, M., Demnerova, K., Heuvelink, A. E., Tassios, P. T., Lindmark, H., Kmet, V., Barbanera, M., Fach, P., Loncarevic, S., & Hoorfar, J. (2004). Validation of a PCR-based method for detection of food-borne thermotolerant Campylobacters in a multicenter collaborative trial. *Applied and Environmental Microbiology, 70*(7), 4379–4383. https://doi.org/10.1128/AEM.70.7.4379

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews, 28*(3), 687–720. https://doi.org/10.1128/CMR.00006-15

Karlsson, O., & Subramanian, S. V. (2023). Refrigerator ownership and child health and nutrition in low- and middle-income countries. *Global Food Security, 37*. https://doi.org/10.1016/j.gfs.2023.100698

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Alter, T., Crotta, M., Ellis-Iversen, J., Hempen, M., Messens, W., & Chemaly, M. (2020). Update and review of control options for *Campylobacter* in broilers at primary production. *EFSA Journal, 18*(4), 1–89. https://doi.org/10.2903/j.efsa.2020.6090

Lindqvist, R., & Lindblad, M. (2008). Quantitative risk assessment of thermophilic *Campylobacter* spp. and cross-contamination during handling of raw broiler chickens evaluating strategies at the producer level to reduce human campylobacteriosis in Sweden. *International Journal of Food Microbiology, 121*(1), 41–52. https://doi.org/10.1016/j.ijfoodmicro.2007.10.008

Lv, R., Wang, K., Feng, J., Heeney, D. D., Liu, D., & Lu, X. (2020). Detection and quantification of viable but non-culturable *Campylobacter jejuni*. *Frontiers in Microbiology, 10*, 1–8. https://doi.org/10.3389/fmicb.2019.02920

Mäesaar, M., Praakle, K., Meremäe, K., Kramarenko, T., Sögel, J., Viltrop, A., Muutra, K., Kovalenko, K., Matt, D., Hörman, A., Hänninen, M. L., & Roasto, M. (2014). Prevalence and counts of *Campylobacter* spp. in poultry meat at retail level in Estonia. *Food Control, 44*, 72–77. https://doi.org/10.1016/j.foodcont.2014.03.044

Magana, M., Chatzipanagiotou, S., Burriel, A. R., & Ioannidis, A. (2017). Inquiring into the gaps of *Campylobacter* surveillance methods. *Veterinary Sciences, 4*(3), 1–14. https://doi.org/10.3390/vetsci4030036

Maziero, M. T., & de Oliveira, T. C. R. M. (2010). Effect of refrigeration and frozen storage on the *Campylobacter jejuni* recovery from naturally contaminated broiler carcasses. *Brasilian Journal of Microbiology, 41*(2), 501–505. https://doi.org/10.1590/S1517-83822010000200034

Ministerio de Desarrollo Agrario y Riego. (2022). Boletín estadístico mensual: Producción y Comercialización de Productos Avícolas. Retrieved from https://sieamidagri.gob.pe/portal/phocadownload/datos_estadisticas/mensual/Avicola/2022/avicola_06_2022.pdf. Accessed October 20, 2024.

[MINSA-PERU] Peruvian-Ministry-of-Health. (2008). Norma Sanitaria que establece los criterios microbiológicos de calidad sanitaria e inocuidad para los alimentos y bebidas de consumo humano. NTS N° 071 - MINSA/DIGESA-V.01.

Mullner, P., Spencer, S. E. F., Wilson, D. J., Jones, G., Noble, A. D., Midwinter, A. C., Collins-Emerson, J. M., Carter, P., Hathaway, S., & French, N. P. (2009). Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach. *Infection, Genetics and Evolution, 9*(6), 1311–1319. https://doi.org/10.1016/j.meegid.2009.09.003

Munayco, C. V., Gavilan, R. G., Ramirez, G., Loayza, M., Miraval, M. L., Whitehouse, E., Gharpure, R., Soares, J., Soplopuco, H. V., & Sejvar, J. (2020). Large outbreak of Guillain-Barré syndrome, Peru, 2019. *Emerging Infectious Diseases, 26*(11), 2778–2780. https://doi.org/10.3201/eid2611.200127

Nastasijevic, I., Proscia, F., Boskovic, M., Glisic, M., Blagojevic, B., Sorgentone, S., Kirbis, A., & Ferri, M. (2020). The European Union control strategy for *Campylobacter* spp. in the broiler meat chain. *Journal of Food Safety, 40*(5). https://doi.org/10.1111/jfs.12819

Nauta, M. J., Jacobs-reitsma, W. F., & Havelaar, A. H. (2007). A risk assessment model for *Campylobacter* in broiler meat. *Risk Analysis, 27*(4), 845–861. https://doi.org/10.1111/j.1539-6924.2006.00834.x

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *ASM Journal, 69*(8), 4343–4351. https://doi.org/10.1128/AEM.69.8.4343-4351.2003

Newell, D. G., Mughini-Gras, L., Kalupahana, R. S., & Wagenaar, J. A. (2017). *Campylobacter* epidemiology-sources and routes of transmission for human infection. In G. Klein (Ed.), *Campylobacter*: Features, Detection, and Prevention of Foodborne Disease (pp. 85–110). Elsevier Inc. https://doi.org/10.1016/B978-0-12-803623-5.00005-8.

Pohlert, T. (2022). PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended (R package version 1.9.6).

Pozza, S., Voss-rech, D., Silveira, C., & Vaz, L. (2020). Research Note : A baseline survey of thermotolerant *Campylobacter* in retail chicken in southern Brazil. *Poultry Science, 99*, 2690–2695. https://doi.org/10.1016/j.psj.2019.12.061

Rasschaert, G., De Zutter, L., Herman, L., & Heyndrickx, M. (2020). *Campylobacter* contamination of broilers: The role of transport and slaughterhouse. *International Journal of Food Microbiology, 322*, Article 108564. https://doi.org/10.1016/j.ijfoodmicro.2020.108564

R-Core-Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Sarjit, A., & Dykes, G. A. (2017). Transfer of *Campylobacter* and *Salmonella* from poultry meat onto poultry preparation surfaces. *Journal of Food Protection, 80*(5), 750–757. https://doi.org/10.4315/0362-028X.JFP-16-414

Sheppard, S. K., & Maiden, M. C. J. (2015). The evolution of *Campylobacter jejuni* and *Campylobacter coli*. *Cold Spring Harbor Perspectives in Biology, 7*(8), 1–14. https://doi.org/10.1101/cshperspect.a018119

Skarp, C. P. A., Hänninen, M. L., & Rautelin, H. I. K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection, 22*(2), 103–109. https://doi.org/10.1016/j.cmi.2015.11.019

Slader, J., Domingue, G., Jørgensen, F., McAlpine, K., Owen, R. J., Bolton, F. J., & Humphrey, T. J. (2002). Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Applied and Environmental Microbiology, 68*(2), 713–719. https://doi.org/10.1128/AEM.68.2.713-719.2002

Stingl, K., Heise, J., Thieck, M., Wulsten, I. F., Pacholewicz, E., Iwobi, A. N., Govindaswamy, J., Zeller-Péronnet, V., Scheuring, S., Luu, H. Q., Fridriksdottir, V., Gölz, G., Priller, F., Gruntar, I., Jorgensen, F., Koene, M., Kovac, J., Lick, S., Répérant, E., & Huber, I. (2021). Challenging the "gold standard" of colony-forming units - Validation of a multiplex real-time PCR for quantification of viable *Campylobacter* spp. in meat rinses. *International Journal of Food Microbiology, 359*. https://doi.org/10.1016/j.ijfoodmicro.2021.109417

Swart, A., Mangen, M-J., Havelaar, A. (2013). Microbiological criteria as a decision tool for controlling *Campylobacter* in the broiler meat chain. In RIVM Letter Report 330331008/2013. Retrieved from https://www.rivm.nl/bibliotheek/rapporten/330331008.pdf. Accessed October 14, 2023.

Thames, H. T., & Sukumaran, A. T. (2020). A review of *Salmonella* and *Campylobacter* in broiler meat: Emerging challenges and food safety measures. *Foods, 9*(6), 776. https://doi.org/10.3390/foods9060776

Thornval, N. R., & Hoorfar, J. (2021). Progress in detection of *Campylobacter* in the food production chain. *Current Opinion in Food Science, 39*, 16–21. https://doi.org/10.1016/j.cofs.2020.12.001

WHO. (2020). *Campylobacter*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/campylobacter. Accessed May 6, 2023.

Zou, G. (2004). A modified Poisson regression approach to prospective studies with binary data. *American Journal of Epidemiology, 159*(7), 702–706. https://doi.org/10.1093/aje/kwh090

II. DETALLES ADICIONALES DE LA METODOLOGÍA

Durante el muestreo de carne de pollo detallado en el artículo publicado, la información sobre las características de la infraestructura y de las condiciones de venta de carne de pollo dentro de los puestos de mercado de abasto fue recolectada mediante un formulario con opciones de respuesta múltiples, desarrollado en la aplicación móvil Epicollect5 (1). En cada opción de respuesta múltiple se describían los criterios a tomar en cuenta para que la colecta de datos fuera lo más uniforme posible. Este formulario fue completado por el personal de campo en el momento de la visita, a partir de observación directa, seleccionando las opciones que correspondían según lo observado en cada puesto. Una descripción detallada del formulario y de las categorías de respuesta se presenta en el Cuadro suplementario S1 del artículo publicado, también incluido en el ANEXO 1 del presente manuscrito.

Mediante el formulario mencionado, se colectó información sobre si el puesto de mercado vendía exclusivamente carne de pollo, si el producto estaba listo para la venta sin necesidad de manipulación adicional, el estado de limpieza de la mesa de corte y del paño de limpieza, así como la disponibilidad de refrigeración y agua corriente. También se consideró la aceptabilidad de la infraestructura general del puesto, definida como la presencia de superficies lavables, piezas organizadas, ausencia de acumulación de vísceras y baja presencia de moscas.

Cada muestra fue almacenada individualmente en bolsas plásticas rotuladas y colocada en cajas térmicas con geles refrigerantes para su transporte inmediato. Las cajas permanecieron cerradas durante el traslado y las muestras no estuvieron en contacto directo con los geles, lo que permitió mantener temperaturas de

refrigeración sin riesgo de congelación. En cada envío se utilizó un registrador de temperatura TempTale® Ultra (Sensitech Inc., Redmond, WA), lo que permitió verificar que las muestras se conservaron en un rango de 3 °C a 8 °C desde su recolección en los mercados de abasto hasta su llegada al laboratorio en Jauja, Junín.

Según la sección 8.2.3.1 del estándar internacional ISO/TS 17728:2017, el transporte de muestras de cuello de aves de corral no requiere un medio específico previo al cultivo, siempre que se mantenga la cadena de frío y se evite la congelación (2). Asimismo, la norma ISO 7218 establece que las muestras pueden conservarse hasta 24 horas a una temperatura de 3 °C ± 2 °C antes del análisis microbiológico sin afectar la viabilidad de los patógenos (3).

En este estudio, todas las muestras, incluidas las provenientes de Tumbes, Piura y Huaral, fueron procesadas dentro de ese plazo. Las condiciones de transporte y conservación fueron idénticas en todas las regiones, con refrigeración controlada y monitoreo continuo de temperatura. Las muestras de regiones distintas a la ubicación del laboratorio (como Huaral y Tumbes/Piura) fueron recolectadas a primera hora de la mañana los días domingo y trasladadas por vía aérea ese mismo día hasta el laboratorio en Jauja, bajo las condiciones ya descritas, para ser analizadas dentro de las 24 horas posteriores a su colecta.

Por ello, la ausencia de aislamientos positivos de *Campylobacter* spp. mediante cultivo bacteriológico en las muestras de Tumbes/Piura no podría atribuirse a fallas logísticas, ya que estas siguieron el mismo protocolo y tiempo de transporte que las de Huaral, donde sí se obtuvieron cultivos positivos. Esta diferencia probablemente se relacione con factores propios de los puntos de muestreo, como las prácticas de

manipulación del producto, condiciones higiénicas o carga bacteriana inicial, aspectos que se abordan más adelante en la sección de discusión de este documento.

Una vez en el laboratorio, además del análisis mediante cultivo bacteriológico, las muestras fueron evaluadas mediante un ensayo de reacción en cadena de la polimerasa cuantitativa en tiempo real (qPCR, por sus siglas en inglés) para la detección y cuantificación de *Campylobacter* spp. termotolerantes. El ensayo de qPCR utilizado fue previamente validado en un estudio colaborativo multicéntrico, en el que participaron 12 laboratorios que evaluaron muestras de enjuague de canales inoculadas experimentalmente (4). En esta evaluación, el método mostró una sensibilidad de 96.7 % y una especificidad del 100 % para muestras de pollo (4).

La cuantificación del número de copias genómicas (CG) se realizó mediante estimación a partir de una curva estándar generada a partir de fragmentos sintéticos de ADN (gBlocks®, Integrated DNA Technologies, Inc., Coralville, IA, USA). La curva se preparó utilizando una serie de diluciones seriadas en base 10 de 12 puntos, que abarcó concentraciones desde 10^{9} hasta 10^{-2} CG. Si bien no se validó un límite de detección (LOD) localmente, se consideró como umbral el punto más bajo de la curva en el que se obtuvo amplificación reproducible con valores de Ct menores a 40. En este caso, se observó amplificación consistente a partir de 10 CG, mientras que a 1 CG no se detectó amplificación, por lo que el LOD operativo del ensayo fue establecido en 10 CG. Las reacciones de qPCR se realizaron por duplicado, y se consideraron positivas aquellas muestras que mostraron amplificación en al menos una réplica dentro del umbral definido.

La evaluación cuantitativa del riesgo microbiológico (QMRA, por sus siglas en inglés) se basó en los resultados de proporción de muestras positivas y cuantificación de *Campylobacter spp.* obtenidos por cultivo bacteriológico, según lo descrito en la sección 2.2 del artículo publicado. Solo se utilizaron resultados de cultivo, ya que el modelo requiere bacterias viables. Para parametrizar el modelo con datos peruanos, se recopilaron variables demográficas y de consumo de carne de pollo a partir de encuestas y censos nacionales. Dado que las regiones muestreadas presentaban diferencias en clima, tamaño poblacional y acceso a refrigeración, se desarrollaron modelos específicos por región (5). Ante la falta de información sobre prácticas de almacenamiento en el hogar y eventos de contaminación cruzada, se simularon distintos escenarios para evaluar su impacto en la exposición.

Es importante precisar que la información recolectada sobre las características de los puestos en mercados de abasto no fue incluida como variable de entrada en el modelo QMRA. Las variables categóricas correspondientes, como “puesto limpio”, “infraestructura aceptable” o “presencia de refrigeración”, se utilizaron únicamente en análisis exploratorios para evaluar su asociación con la presencia y carga de *Campylobacter* spp. detectadas por cultivo y qPCR. Sus definiciones operativas ya fueron descritas al inicio de esta sección y se presentan en detalle en el Cuadro 1 del material suplementario del artículo publicado, también incluido en los anexos del presente manuscrito.

III. DISCUSIÓN

El presente estudio describió el impacto que tendría la presencia y cuantificación de *Campylobacter* spp. en carne de pollo vendida en mercados de abasto de las provincias Huancayo, Huaral, Tumbes y Piura. Se resalta el alarmante nivel de contaminación encontrado, muy por encima de la normativa sanitaria peruana (6). Asimismo, se evaluó el uso potencial de la qPCR como herramienta para la vigilancia rutinaria de *Campylobacter* en complemento al actual método gold estándar, el cultivo bacteriológico. Finalmente, se llevó a cabo un QMRA para estimar el número de casos anuales de campilobacteriosis humana basados en los niveles de contaminación reportados en este estudio y para evaluar estrategias de control dirigidas al nivel consumidor.

2.1 *Campylobacter spp.* en carne de pollo de mercados de abasto

En Perú, el pollo es un componente esencial de la dieta diaria y la principal fuente de proteína animal para la población, representando más del 60% del consumo total de proteínas animales, según el Ministerio de Desarrollo Agrario y Riego (MIDAGRI) (7). Esta dependencia del pollo como alimento básico resalta la importancia de los resultados obtenidos en este estudio, que evidencian elevados niveles de *Campylobacter*, una bacteria patógena vinculada con intoxicaciones alimentarias y enfermedades gastrointestinales (8).

En particular, reportamos una cuantificación promedio de *Campylobacter* spp. de 3.31 $\log_{10}$ unidades formadoras de colonias por gramo de pollo (UFC/g) en las muestras contaminadas. Esta cifra revela un elevado nivel de contaminación en la carne de pollo vendida para consumo en mercados de abasto de las ciudades evaluadas, específicamente en Huancayo y Huaral. Este nivel de contaminación está

en línea con reportes internacionales de países como Brasil y Estonia, aunque supera las cuantificaciones reportadas en Australia y Lituania (9–12). Por lo que, considerando el alto consumo de pollo en el país y su presencia constante en los hogares peruanos, estos hallazgos podrían tener serias implicaciones para la salud pública. Esto subraya la necesidad de evaluar estrategias de control y mitigación, no solo a nivel de mercados de abasto, sino también a lo largo de la cadena de producción, comercialización y en el nivel consumidor.

Por otro lado, en el estudio se reporta que todas las muestras de carne de pollo provenientes de las provincias de Tumbes y Piura, pertenecientes a las regiones homónimas, resultaron negativas para *Campylobacter* spp. mediante cultivo. Sin embargo, la detección y cuantificación de material genético de *Campylobacter* mediante qPCR en estas regiones sugiere la posible presencia de bacterias no viables, en estado viable pero no cultivable, o en concentraciones inferiores al límite de detección del método de cultivo (13). Dado el número de observaciones disponibles en ambas provincias, así como las similitudes en características climáticas, condiciones de los mercados y origen de la carne de pollo destinada a la venta, se decidió agrupar las provincias bajo la denominación Tumbes/Piura para fines analíticos en este estudio.

En el estudio se discuten los posibles factores que pudieron influenciar en la ausencia de muestras contaminadas con *Campylobacter* spp. en Tumbes/Piura. Entre ellos, la ventilación controlada en las granjas que contrarestan las altas temperaturas de esta región, combinada con el uso más frecuente de refrigeración en los mercados, podría explicar estas diferencias (14). Además, es posible que algunos vendedores comercialicen pollo previamente congelado como si fuera

fresco, buscando evitar su rápida descomposición bajo altas temperaturas. En este sentido, es importante considerar que el congelamiento, aunque efectivo para reducir las concentraciones bacterianas, puede inducir estados de viabilidad no cultivable, complicando la detección y cuantificación de *Campylobacter* mediante cultivo bacteriológico (13).

Sin embargo, si bien en este estudio no se reportó *Campylobacter* en Tumbes/Piura, consideramos que se deberían seguir monitoreando estas provincias. Esto debido a que, en los últimos años, se ha reportado un aumento en los casos de síndrome de Guillain-Barré (SGB) en la región norte del país (15). En el año 2023, un año después de la recolección de las muestras de pollo analizadas en el estudio madre, el Centro Nacional de Epidemiología, Prevención y Control de Enfermedades emitió una alerta epidemiológica debido al aumento de casos del SGB en varias ciudades del país, incluida Piura (16). En dicha alerta, se enfatizó la importancia de fortalecer la vigilancia epidemiológica de los posibles desencadenantes de este trastorno neurológico. Además, se destacó la necesidad de comunicar mensajes clave a la población sobre la correcta manipulación de alimentos y la implementación de medidas efectivas para evitar la contaminación cruzada durante su almacenamiento y preparación (16).

2.2 Adaptación de la normativa peruana a los estándares europeos frente a *Campylobacter spp.*

El control de *Campylobacter spp.* en la cadena de producción de carne de pollo es un desafío global debido a su ubicuidad y alta prevalencia en las aves de corral y se ha reportado que aún no es posible lograr su eliminación total en las granjas de producción (17). En este sentido, la normativa europea, establece un límite máximo

de 1000 UFC/g para que la carne de pollo sea considerada apta para su comercialización (18). Este enfoque se fundamenta en el reconocimiento de la imposibilidad de erradicar completamente el patógeno y en la necesidad de mitigar el riesgo asociado a su presencia. Se ha demostrado que la implementación de este límite de contaminación puede reducir los casos de campilobacteriosis humana hasta en dos tercios (19).

Por otro lado, en Perú, la ausencia de una regulación específica para *Campylobacter* spp. representa un vacío crítico en la vigilancia de la seguridad alimentaria. La normativa actual, que establece criterios microbiológicos para la inocuidad alimentaria, se limita a requerir la ausencia de microorganismos patógenos, considerando cualquier presencia de estos como un riesgo para la salud (6). Sin embargo, los hallazgos de este estudio revelan niveles de contaminación que superan incluso los límites establecidos por la normativa europea (18). Esto evidencia la necesidad de revisar y especificar los parámetros en la normativa vigente, ajustando el límite permitido a un valor más pragmático, como el europeo. Implementar un estándar de este tipo fortalecería el sistema de vigilancia alimentaria peruano, facilitando un control más efectivo del riesgo y alineando al país con prácticas internacionales de seguridad alimentaria.

2.3 Factores de riesgo para la presencia de *Campylobacter* spp. asociados a las características de los mercados de abasto

Los mercados de abasto constituyen la principal vía de comercialización de carne de pollo fresca destinada al consumo en los hogares peruanos (7). Este estudio identificó los factores de riesgo presentes en los puestos de mercado que podrían

abordarse para reducir la prevalencia y carga de *Campylobacter* en la carne de pollo y proteger la salud de los consumidores.

Además del análisis de regresión de Poisson modificado presentado en el artículo publicado, en el que se muestran los resultados crudos y ajustados por región, se exploró un modelo múltiple ajustado simultáneamente por todas las características de los puestos evaluadas y por región. Los resultados de este análisis adicional se presentan en el ANEXO 2, donde se observa que la presencia de refrigeradora y de agua corriente dentro del puesto se asoció significativamente con la detección de *Campylobacter*. Esto contrasta con el modelo ajustado únicamente por región, en el que solo el acceso a agua corriente fue estadísticamente significativo en las muestras positivas por cultivo. Este hallazgo es consistente con el análisis bivariado incluido en el artículo publicado, en el que ambos factores también mostraron una relación significativa con las cuantificaciones obtenidas tanto por cultivo como por qPCR.

En este sentido, uno de los factores asociados a una mayor cuantificación encontrada de *Campylobacter* es la falta de refrigeradoras en los puestos de venta. Al no contar con este electrodoméstico, la carne de pollo se mantiene a temperatura ambiente durante varias horas, lo que podría incrementar el riesgo de transmisión al consumidor (20,21). *Campylobacter spp.* prospera a temperaturas entre 30 °C y 45 °C, y aunque no crece por debajo de 4 °C, puede sobrevivir durante periodos prolongados en condiciones subóptimas (22). Este problema se agrava durante los meses más cálidos del año o en mercados sin una adecuada ventilación, cuando las temperaturas ambientales elevadas aceleran el deterioro microbiológico de los alimentos. A mediados de 2023, el Ministerio de Salud introdujo una norma

sanitaria que regula las condiciones de los mercados de abasto, estableciendo que todos los puestos de venta de carnes deben disponer de medios de refrigeración o congelación (6). En nuestro estudio, que emplea datos de hasta seis meses antes de la entrada en vigor de esta norma, solo el 21 % (19/90) de los puestos analizados disponía de refrigeradoras. Esto resalta la importancia de monitorear si los mercados cumplen actualmente con la normativa y, de no ser así, reforzar las medidas para garantizar su implementación.

Otro factor discutido en el estudio es la disponibilidad de agua corriente dentro de los puestos del mercado de abasto. Si bien el acceso al agua es una necesidad básica para la higiene, su uso inapropiado podría incrementar el riesgo de contaminación cruzada, especialmente cuando se emplea para lavar carne contaminada o utensilios sin protocolos adecuados de separación y desinfección (23,24). Si los comerciantes lavaran la carne de pollo para eliminar restos orgánicos y hacerla visualmente más atractiva, podrían estar favoreciendo la diseminación de *Campylobacter* a través de salpicaduras y contacto con superficies contaminadas (24). El lavado de pollo crudo no reduce la carga bacteriana y las salpicaduras generadas durante el proceso pueden alcanzar distancias de hasta 70 cm (25). Esta práctica, común en hogares peruanos, no solo incrementa el riesgo de exposición directa al patógeno, sino que también compromete la seguridad de otros productos presentes en el mismo entorno (26).

Esta situación se ve agravada por el hecho de que *Campylobacter* tiene la capacidad de formar biopelículas sobre superficies inertes, lo que dificulta su eliminación durante la limpieza (27,28). En mercados de abasto con bajo control sanitario, el uso repetido del agua para diversas actividades, la falta de separación entre áreas

limpias y sucias, el escaso uso de desinfectantes y la atención continua a múltiples clientes pueden convertir el agua corriente en un vehículo de transmisión más que en una medida de control. Por ello, como recomendación basada en los hallazgos del estudio, se sugiere la implementación de programas de capacitación para los vendedores, enfocados en mejorar las prácticas de manejo y reducir la contaminación cruzada (26).

2.4 Uso potencial de qPCR como herramienta en la vigilancia rutinaria de *Campylobacter*

La qPCR es una herramienta prometedora en la vigilancia de *Campylobacter*, con estudios previos que sugieren que podría ser tan eficaz como los métodos de cultivo (29). Sin embargo, a diferencia del cultivo bacteriológico, que permite evaluar la viabilidad del microorganismo, los ensayos de qPCR no siempre son capaces de distinguir entre material genético viable e inactivo o no cultivable (30). En este estudio, empleamos un ensayo de qPCR que nos permitió detectar y cuantificar el material genético de *Campylobacter*, independientemente de su viabilidad (4), lo que implica que los hallazgos de copias genómicas por gramo de pollo (CG/g) no pueden ser interpretados de la misma manera que los hallazgos de unidades formadoras de colonias por gramo (UFC/g), ni reflejan necesariamente un riesgo real para la población.

A pesar de esta limitación, los resultados del análisis de la curva de características operativas del receptor (ROC, por sus siglas en inglés) ofrecen una vía para utilizar la qPCR como una herramienta de tamizaje rápida. En este análisis, se identificó un punto de corte de 5.37 $\log_{10}$ GC/g con una especificidad del 85% (IC 95%: 0.74-0.92) para predecir muestras negativas en cultivo. Este umbral podría

ser útil para evaluar rápidamente lotes de carne de pollo potencialmente libres de *Campylobacter*, mientras se lleva a cabo el largo proceso de cultivo bacteriológico. Sin embargo, antes de su implementación generalizada, es necesario validar este punto de corte para garantizar que su uso complementario con el cultivo sea eficaz en la mejora de la seguridad alimentaria, permitiendo una vigilancia más rápida y oportuna.

2.5 Hallazgos del QMRA y evaluación de estrategias de control

El QMRA realizado en este estudio reveló un riesgo elevado de adquirir campilobacteriosis en Perú a partir del consumo de carne contaminada, estimado en 600 veces más alto que el reportado en los Países Bajos (31). Este resultado no solo se debería a las diferencias en prevalencia y carga bacteriana entre ambos países, sino también a las características estructurales y culturales de la cadena alimentaria (5,7). Aunque las simulaciones sugieren que cada consumidor de las poblaciones evaluadas de Perú podría desarrollar campilobacteriosis anualmente bajo parámetros conservadores, este análisis no se enfoca en discutir y comparar las cifras simuladas con las reportadas en el país. Esto se debe a que no podemos determinar si estas cifras estarían sobreestimadas o subestimadas, debido a las limitaciones en el sistema de vigilancia de *Campylobacter* en Perú y la falta de caracterización del agente en los brotes de gastroenteritis. Por lo tanto, el estudio se enfocó en la evaluación de estrategias de control y cómo su implementación podría contribuir a la reducción de los casos simulados de campilobacteriosis humana.

Además, se detallan estrategias de control de la campilobacteriosis que consideramos viables para su implementación en Perú, al no requerir la necesidad de grandes inversiones en infraestructura. La reducción de la contaminación

cruzada a nivel del consumidor podría reducir hasta en un 75 % los casos de campilobacteriosis humana simulados, mientras que aumentar la proporción de la población con refrigeradoras que almacena el pollo a temperaturas de congelación podría reducir los casos en un 40%. Estas acciones podrían implementarse mediante campañas educativas, aprovechando la infraestructura existente y sin la necesidad de adquirir o distribuir nuevos equipos. Las campañas podrían enfocarse en sensibilizar a la población sobre los peligros de la contaminación cruzada en las cocinas, el uso de utensilios diferentes para alimentos crudos y cocidos, y el riesgo de lavar la carne de pollo (32,33). En cuanto al almacenamiento de la carne, se orientaría a los consumidores sobre los beneficios de la congelación previa al consumo (34,35), promoviendo hábitos más seguros, especialmente en las zonas rurales, donde la infraestructura de refrigeración puede ser limitada.

No obstante, se debe considerar que la efectividad de las campañas educativas dependerá de la capacidad de adaptar los mensajes a las realidades de las zonas rurales y urbanas, donde los hábitos alimentarios y las condiciones de almacenamiento pueden variar considerablemente. Además, aunque la educación sobre la contaminación cruzada podría reducir los riesgos de manera importante, su implementación requiere un cambio de paradigma en las prácticas cotidianas, lo que puede ser difícil sin un acompañamiento continuo. Por lo que el éxito de estas estrategias dependerá de un esfuerzo coordinado entre los diferentes actores del sistema alimentario, incluidos los productores, distribuidores, autoridades sanitarias y los propios consumidores.

Por otro lado, se evaluó cómo la reducción de la prevalencia de carne de pollo contaminada en los mercados influiría en la disminución de los casos simulados de

campilobacteriosis humana. Sin embargo, la prevalencia de *Campylobacter* en los lotes de pollo no depende únicamente de las medidas de bioseguridad implementadas en las granjas, sino también de las condiciones de transporte, procesamiento y distribución (28). Aunque mejorar los estándares de bioseguridad en las granjas puede reducir la contaminación inicial, esta puede persistir en etapas posteriores de la cadena de producción (17). Por lo tanto, para lograr una reducción de la prevalencia de *Campylobacter* en el pollo comercializado en los mercados de abasto, es necesario adoptar una estrategia integral que abarque desde las granjas de producción hasta las plantas de procesamiento, el transporte y los mercados de abasto.

Finalmente, se discuten las posibles limitaciones del estudio que deben ser consideradas en la interpretación de los resultados. Una de las principales limitaciones es que no se diferenciaron las especies de *Campylobacter* en el estudio primario. Esto impide conocer la distribución de especies en las regiones evaluadas, lo cual limita la extrapolación de los resultados. Especies de *Campylobacter* podrían diferir en su virulencia, frecuencia en humanos y carga de enfermedad, por lo que asumir un comportamiento homogéneo podría afectar las estimaciones del modelo.

Por ejemplo, *C. jejuni* causa aproximadamente el 90% de las infecciones humanas, mientras que *C. coli* representa menos del 10% (36–38). Este menor impacto en salud pública se relaciona con la ausencia de varios factores de virulencia en *C. coli*, lo cual ha sido confirmado por análisis genómicos que evidencian la falta de genes asociados a la patogenicidad presentes en *C. jejuni* (39,40). Y aunque ambas especies pueden ocasionar infecciones más allá del tracto digestivo, *C. jejuni* se asocia más frecuentemente con secuelas como el SGB, artritis

reactiva y síndrome urémico hemolítico (41). Esta limitación no solo afecta el estudio, sino también refleja una debilidad del sistema de vigilancia en Perú, que no contempla la identificación a nivel de especie.

Además, esta falta de diferenciación entre especies es especialmente relevante porque el modelo QMRA empleado fue parametrizado utilizando una curva dosis-respuesta específica para *C. jejuni*, construida a partir de estudios experimentales en humanos realizados en Baltimore, EE. UU. (42,43). Esta curva corresponde a una distribución beta-binomial, cuyos parámetros alpha y beta reflejan la variabilidad en la susceptibilidad individual y permiten estimar la probabilidad de infección según la dosis ingerida. Si bien esta función representa una propiedad biológica del patógeno y es ampliamente aceptada en evaluaciones de riesgo microbiológico, podría no describir adecuadamente la respuesta infecciosa de otras especies como *C. coli*. Por tanto, si *C. coli* fuera más predominante en las regiones evaluadas, el uso exclusivo de parámetros de *C. jejuni* podría llevar a una sobreestimación o subestimación de los casos simulados.

Aunque el modelo sQMRA fue desarrollado originalmente en los Países Bajos, este estudio no incorporó ninguno de los escenarios, supuestos ni parámetros específicos del contexto neerlandés. Todos los parámetros que son afectados por el contexto local, como condiciones de almacenamiento y preparación, prácticas de consumo y características de la población, fueron obtenidos exclusivamente de encuestas nacionales de Perú. No obstante, una limitación es que el modelo no considera la inmunidad adquirida por exposiciones previas a *Campylobacter*, lo que podría afectar la probabilidad real de infección en poblaciones altamente expuestas. Se ha reportado que la exposición temprana al patógeno puede inducir una

inmunidad protectora, por lo que su omisión podría llevar a una sobreestimación de los casos simulados (44).

Finalmente, los resultados generados por el modelo sQMRA son datos *in silico* que ofrecen aproximaciones útiles para la toma de decisiones, pero tienen limitaciones inherentes, especialmente la necesidad de validación en contextos reales, lo cual suele ser costoso e impráctico. Esto puede limitar la aplicabilidad de las estrategias de control derivadas de los resultados del modelo, por lo que es necesario contar con datos más específicos sobre factores como la contaminación cruzada y el almacenamiento en los consumidores de Perú para obtener estimaciones más precisas.

IV. REFERENCIAS BIBLIOGRÁFICAS

1. Gupta S, Sharma S, Gohil R, Sachdeva S. Epicollect5: a free, fully customizable mobile-based application for data collection in clinical research. J Postgrad Med Educ Res. 2021;54(4):248–51.
2. [ISO] International Organization for Standardizarion 17728:2017. Microbiology of food and animal feed - Sampling techniques for microbiological analysis of food and feed samples. 2017.
3. [ISO] International Organization for Standardizarion 7218:2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations. 2007.
4. Josefsen MH, Cook N, Agostino MD, Hansen F, Wagner M, Demnerova K, et al. Validation of a PCR-based method for detection of food-borne thermotolerant Campylobacters in a multicenter collaborative trial. Appl Env Microbiol. 2004;70(7):4379–83.
5. [INEI] Instituto Nacional de Estadística e Informática. Censos Nacionales 2017: XII de Población, VII de Vivienda y III de Comunidades Indígenas [Internet]. Lima: INEI; 2017 [citado en 11 de noviembre de 2023]. Disponible en: https://censo2017.inei.gob.pe/resultados-definitivos-de-los-censos-nacionales-2017/
6. [MINSA-PERU] Ministerio de Salud de Perú. Norma Sanitaria que establece los criterios microbiológicos de calidad sanitaria e inocuidad para los alimentos y bebidas de consumo humano. Lima: Minsa; 2008. 28 p. NTS N° 071 - MINSA/DIGESA-V.01.

7. Ministerio de Desarrollo Agrario y Riego. Boletín estadístico mensual: Producción y Comercialización de Productos Avícolas [Internet]. Lima: MIDAGRI; 2022 [citado 20 de octubre de 2024]. Disponible en: https://siea.midagri.gob.pe/portal/phocadownload/datos_estadisticas/mensual/Avicola/2022/avicola_06_2022.pdf

8. WHO. *Campylobacter* [Internet]. 2020 [citado 6 de mayo de 2024]. Disponible en: https://www.who.int/news-room/fact-sheets/detail/campylobacter

9. Pozza S, Voss-rech D, Silveira C, Vaz L. Research Note : A baseline survey of thermotolerant *Campylobacter* in retail chicken in southern Brazil. Poultry Science. 2020; 99: 2690–2695. https://doi.org/10.1016/j.psj.2019.12.061

10. Mäesaar M, Praakle K, Meremäe K, Kramarenko T, Sõgel J, Viltrop A, Muutra K, Kovalenko K, Matt D, Hörman A, Hänninen ML, Roasto M. Prevalence and counts of *Campylobacter* spp. in poultry meat at retail level in Estonia. Food Control. 2014; 44: 72–77. https://doi.org/10.1016/j.foodcont.2014.03.044

11. Habib I, Coles J, Fallows M, Goodchild S. A baseline quantitative survey of *Campylobacter* spp. on retail chicken portions and carcasses in Metropolitan Perth, Western Australia. Foodborne Pathogens and Disease. 2018; 16(3): 180–186. https://doi.org/10.1089/fpd.2018.2554

12. Bunevičienė J, Kudirkienė E, Ramonaite S, Malakauskas M. Occurrence and numbers of *Campylobacter* spp. on wings and

drumsticks of broiler chickens at the retail level in Lithuania. Vet Med Zoot. 2010;50(72):9–14.

13. Lv R, Wang K, Feng J, Heeney DD, Liu D, Lu X. Detection and quantification of viable but non-culturable *Campylobacter jejuni*. Front Microbiol. 2020;10:1–8. https://doi.org/10.3389/fmicb.2019.02920

14. Instituto Nacional de Estadística e Informática. Perú: Anuario de Estadísticas Ambientales 2022 [Internet]. Lima: INEI; 2022 [citado 15 de diciembre de 2023]. Disponible en: https://www.inei.gob.pe/media/MenuRecursivo/publicaciones_digitales/Est/Lib1877/libro.pdf

15. CDC MINSA. Situación epidemiológica del Síndrome de Guillain-Barré en el Perú SE 04-2022 [Internet]. Lima: CDC; 2022. [citado 2 de julio de 2023]. Disponible en: https://www.dge.gob.pe/epipublic/uploads/boletin/boletin_20214_16_203924_2.pdf

16. CDC MINSA. Alerta epidemiologica del síndrome de Guillain-Barré [Internet]. Lima: CDC; 2023. [citado 14 de diciembre de 2023]. Disponible en: https://www.dge.gob.pe/epipublic/uploads/alertas/alertas_202315_26_141558.pdf

17. Georgiev M, Beauvais W, Guitian J. Effect of enhanced biosecurity and selected on-farm factors on Campylobacter colonization of chicken broilers. Epidemiol Infect. 2017;145(3):553–567. https://doi.org/10.1017/S095026881600251X

18. European Commission. Commission Regulation (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcasses [Internet]. Brucelas: European Commission; 2017 [citado 26 de abril de 2023]. Disponible en: http://data.europa.eu/eli/reg/2017/1495/oj.

19. Swart A, Mangen M-J, Havelaar A. Microbiological criteria as a decision tool for controlling *Campylobacter* in the broiler meat chain. En RIVM Letter Report 330331008/2013 [Internet]. 2013. [citado 14 de octubre de 2023]. Disponible en: https://www.rivm.nl/bibliotheek/rapporten/330331008.pdf

20. Endtz HP. *Campylobacter* Infections. En: Ryan ET, Hill DR, Solomon T, Aronson NE, Endy TP, editors. Hunter's Tropical Medicine and Emerging Infectious Disease. 10th ed. Elsevier Inc; 2019. p. 507–511. https://doi.org/10.1016/B978-0-323-55512-8.00050-8

21. Fitzgerald C. *Campylobacter*. Clin Lab Med. 2015;35:289–298. https://doi.org/10.1016/j.cll.2015.03.001

22. [ISO]. International Organization for Standardization 10272-2: 2017. Microbiology of the food chain - horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique. 2017. 19 p.

23. Habib I, Harb A, Hansson I, Vågsholm I, Osama W, Adnan S, Anwar M, Agamy N, Boqvist S. Challenges and opportunities towards the development of risk assessment at the consumer phase in developing countries—The case of *Campylobacter* cross-contamination during

handling of raw chicken in two middle eastern countries. Pathogens. 2020;9(1):62. https://doi.org/10.3390/pathogens9010062

24. Sarjit A, Dykes GA. Transfer of *Campylobacter* and *Salmonella* from poultry meat onto poultry preparation surfaces. J Food Prot. 2017;80(5):750–757. https://doi.org/10.4315/0362-028X.JFP-16-414

25. Everis L, Betts G. Microbial risk factors associated with the domestic handling of meat: sequential transfer of bacterial contamination. Gloucestershire (UK): Campden & Chorleywood Food Research Association Group; 2003.

26. Henley SC, Gleason J, Quinlan JJ. Don't wash your chicken!: A food safety education campaign to address a common food mishandling practice. Food Prot Trends. 2016;36(1):43–53.

27. Lamas A, Regal P, Vásquez B, Miranda J, Cepeda A, Franco C. *Salmonella* and *Campylobacter* biofilm formation: a comparative assessment from farm to fork. J Sci Food Agric. 2018;98(11):4014–402. https://doi.org/10.1002/jsfa.8945

28. Newell DG, Mughini-Gras L, Kalupahana RS, Wagenaar JA. *Campylobacter* epidemiology—sources and routes of transmission for human infection. In: Klein G, editor. *Campylobacter: Features, Detection, and Prevention of Foodborne Disease*. Elsevier Inc.; 2017. p. 85–110. https://doi.org/10.1016/B978-0-12-803623-5.00005-8

29. Stingl K, Heise J, Thieck M, Wulsten IF, Pacholewicz E, Iwobi AN, Govindaswamy J, Zeller-Péronnet V, Scheuring S, Luu HQ, Fridriksdottir V, Gölz G, Priller F, Gruntar I, Jorgensen F, Koene M,

Kovac J, Lick S, Répérant E, Huber I. Challenging the “gold standard” of colony-forming units - Validation of a multiplex real-time PCR for quantification of viable *Campylobacter* spp. in meat rinses. Int J Food Microbiol. 2021;359. https://doi.org/10.1016/j.ijfoodmicro.2021.109417

30. Magana M, Chatzipanagiotou S, Burriel AR, Ioannidis A. Inquiring into the gaps of *Campylobacter* surveillance methods. Vet Sci. 2017;4(3):1–14. https://doi.org/10.3390/vetsci4030036

31. Chardon JE, Evers EG. Improved swift Quantitative Microbiological Risk Assessment (sQMRA) methodology. Food Control. 2017;73:1285–1297. https://doi.org/10.1016/j.foodcont.2016.10.049

32. Lindqvist R, Lindblad M. Quantitative risk assessment of thermophilic *Campylobacter* spp. and cross-contamination during handling of raw broiler chickens evaluating strategies at the producer level to reduce human campylobacteriosis in Sweden. Int J Food Microbiol. 2008;121(1):41–52. https://doi.org/10.1016/j.ijfoodmicro.2007.09.012

33. Cardoso MJ, Ferreira V, Truninger M, Maia R, Teixeira P. Cross-contamination events of *Campylobacter* spp. in domestic kitchens associated with consumer handling practices of raw poultry. Int J Food Microbiol. 2021;338:108984. https://doi.org/10.1016/j.ijfoodmicro.2020.108984

34. Georgsson F, Orkelsson ÁE, Geirsdóttir M, Reiersen J, Stern NJ. The influence of freezing and duration of storage on *Campylobacter* and

indicator bacteria in broiler carcasses. Food Microbiol. 2006;23(7):677–683. https://doi.org/10.1016/j.fm.2005.10.003

35. Maziero MT, de Oliveira TCR. Effect of refrigeration and frozen storage on the *Campylobacter jejuni* recovery from naturally contaminated broiler carcasses. Braz J Microbiol. 2010;41(2):501–505. https://doi.org/10.1590/S1517-83822010000200034

36. Ministry of Health Singapore. Communicable diseases surveillance Singapore 2018. 2019.

37. Public Health Agency of Canada. FoodNet Canada annual report 2018. 2019.

38. European Food Safety Authority. The European union one health 2020 zoonoses report. Finland's Annual Zoonoses Reports. 2021. https://doi.org/10.2903/j.efsa.2021.6971

39. An JU, Lee J, Cho S, Song H. Comparative Genomic Analysis of Livestock-Derived Campylobacter jejuni: Antimicrobial Resistance, Virulence, Mobile Genetic Elements, and Genetic Relatedness. J Microbiol Biotechnol. 2025;35:1–15. https://doi=10.4014/jmb.2411.11044

40. Allos BM. *Campylobacter jejuni* infections: Update on emerging issues and trends. Clin Infect Dis. 2001;32(8):1201–6. https://doi.org/10.1086/319760

41. Igwaran A, Okoh AI. Human campylobacteriosis: A public health concern of global importance. Heliyon. 2019;5(11):e02814. https://doi.org/10.1016/j.heliyon.2019.e02814

42. Teunis PF, Nagelkerke NJ, Haas CN. Dose response models for infectious gastroenteritis. Risk Anal. 1999;19(6):1251–1260. https://doi.org/10.1023/a:1007055316559

43. Teunis PF, Van der Heijden OG, Van der Giessen JWB, Havelaar AH. The dose-response relation in human volunteers for gastro-intestinal pathogens. Bilthoven: RIVM report 284550002; 1996.

44. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of campylobacter infection. Clin Microbiol Rev. 2015;28(3):687–720. https://doi.org/10.1128/CMR.00006-15

V. ANEXOS

ANEXO 1. Material suplementario del artículo publicado:

Cuadro S1. Descripción del formulario creado en el software Epicollect5 sobre la infraestructura y las condiciones higiénicas de los puestos de mercado muestreados.

PREGUNTAS	RESPUESTAS POSIBLES	DESCRIPCIÓN
1) ¿Son aceptables la infraestructura y la higiene del puesto muestreado?	A) Sí	Superficies del puesto hechas de material fácilmente lavable (loza o melamina), piezas de pollo ordenadas y separadas de las vísceras, con poca o nula presencia de moscas y mantenidas limpias.
	B) No	Superficies del puesto hechas de material poroso (madera o triplay), piezas de pollo y vísceras amontonadas, presencia regular de moscas y limpieza no mantenida.
2) ¿El puesto muestreado vende únicamente carne de pollo?	A) Sí	El puesto vende solo carne de pollo.
	B) No	El puesto vende otras carnes además de pollo, como res, cerdo, cordero, etc.
3) ¿La muestra está lista para la venta (es decir, no requiere corte ni manipulación)?	A) Sí	La muestra estaba lista para la venta.
	B) No	La muestra no estaba lista y requirió cortes y manipulaciones adicionales.
4) ¿Cuál es el grado de limpieza de la mesa donde se corta la carne?	A) Limpio	Recién lavada. No se observan residuos de sangre, carne o vísceras.
	B) Moderadamente limpio	Se observa una cantidad mínima de residuos de sangre, carne o vísceras.
	C) Sucio	Se observan residuos de varios cortes. Se ha utilizado en múltiples ocasiones sin lavado.
5) ¿Cuál es el grado de limpieza del paño de limpieza utilizado en las superficies donde se corta y/o coloca la carne?	A) Limpio	Recién lavado. No se observan manchas de sangre, carne ni vísceras.
	B) Moderadamente limpio	Presenta manchas mínimas de sangre, carne o vísceras.
	C) Sucio	Es evidente que ha sido utilizada varias veces sin lavarse.
6) ¿El puesto cuenta con una refrigeradora para almacenar la carne?	A) Sí	Hay una refrigeradora en funcionamiento en el puesto para el almacenamiento de carne.
	B) No	No hay una refrigeradora en funcionamiento en el puesto.

7) ¿El puesto cuenta con agua proveniente de un caño?	A) Sí	El puesto cuenta con suministro de agua a través de un caño.
	B) No	El puesto no cuenta con suministro de agua a través de un caño. Utiliza baldes para almacenar agua.

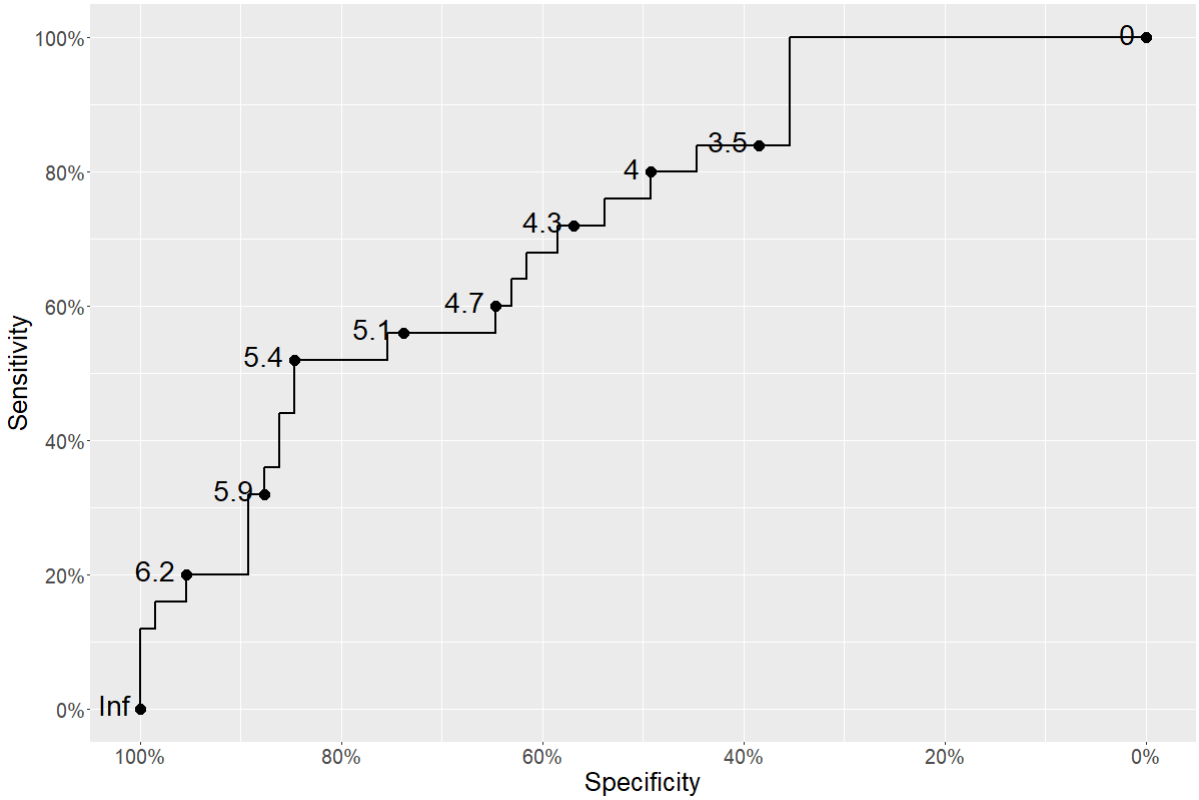

Figura S1. Curva ROC (Receiver Operating Characteristic). La curva ROC fue generada a partir de la cuantificación de Campylobacter (GC/g) en muestras de pollo de mercados tradicionales utilizando qPCR. Al maximizar la suma de sensibilidad y especificidad, se estableció un punto de corte óptimo de 5.37 $\log_{10}$ GC/g, en función de los resultados de cultivo.

ANEXO 2: Razones de prevalencia de las características del puesto para la presencia de *Campylobacter* spp. por cultivo calculado usando el análisis de regresión de Poisson modificada.

Variables	Grupos		Modelo simple		Modelo múltiple**	
	Positivo (n=25)	Negativo (n=65)	RP (IC 95%)	Valor de P	RP (IC 95%)	Valor de P
Infraestructura e higiene aceptables						
Sí	28,9% (22)	71,1% (54)	Ref		Ref	
No	21,4% (3)	78,6% (11)	0,74 (0,25 – 2,17)	0,583	0,56 (0,19 – 1,66)	0,296
El puesto solo vende carne de pollo						
Sí	33,3% (25)	66,7% (50)	Ref		Ref	
No	0,0% (0)*	100,0% (15)	0,20 (0,03 – 1,40)	0,104	0,63 (0,09 – 4,65)	0,649
La muestra estaba lista para vender						
Sí	35,5% (11)	64,5% (20)	Ref		Ref	
No	23,7% (14)	76,3% (45)	0,67 (0,34 – 1,30)	0.237	0,90 (0,49 – 1,64)	0,722
Grado de limpieza de la tabla donde la carne es cortada						
Limpio	12,5% (2)	87,5% (14)	Ref		Ref	
Moderadamente limpio	25,8% (8)	74,2% (23)	2,07 (0,48 – 8,82)	0,328	1,43 (0,24 – 8,65)	0,699
Sucio	34,9% (15)	65,1% (28)	2,79 (0,70 – 11,12)	0,146	0,61 (0,08 – 4,74)	0,639
Grado de limpieza del paño utilizado en las superficies del puesto						
Limpio	12,5% (2)	87,5% (14)	Ref		Ref	
Moderadamente limpio	27,3% (12)	72,7% (32)	2,18 (0,53 – 8,91)	0,277	2,79 (0,36 – 21,85)	0,328
Sucio	36,7% (11)	63,3% (19)	2,93 (0,72 – 11,93)	0,133	4,48 (0,48 – 42,18)	0,190
El puesto cuenta con refrigerador para almacenar la carne						

Sí	22,5% (16)	77,5% (55)	Ref		Ref	
No	47,4% (9)	52,6% (10)	2,10 (1,10 – 4,03)	0,025	2,26 (1,01 – 5,06)	0,047
El puesto cuenta con agua corriente						
No	18,2% (8)	81,8% (36)	Ref		Ref	
Sí	37,0% (17)	63,0% (29)	2,03 (0,97 – 4,26)	0,060	3,90 (1,23 – 12,29)	0,020

RP, Razón de prevalencia; IC 95%, Intervalo de Confianza al 95%

* El valor 0 fue cambiado aleatoriamente a 1 para calcular la razón de prevalencia presentada.

** Modelo múltiple incluyendo las covariables presentadas en la tabla y la variable región utilizando un análisis de regresión de Poisson modificada.