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Presence of *Campylobacter* spp in raw chicken meat. According to its origin and sold in markets in the city of La Paz, Bolivia

Presencia de *Campylobacter* spp. en carne cruda de pollo. Según su procedencia y expendido en mercados de la ciudad de La Paz, Bolivia

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Abstract

Campylobacter spp. is considered a zoonotic agent of worldwide distribution, one of the main reservoirs being poultry. The objective of this research was to detect this agent in raw chicken meat, taking into account the origin, mode of sale, and type of market in the city of La Paz. To detect the microorganism, 108 samples were taken, applying the analytical method ISO 10272-1:2017 Part 1: Detection method. The research was cross-sectional and observational, and Microsoft Excel 2010 and InfoStat (2014) were used for statistical analysis. Data per factor were analyzed with the χ^2 test, with a significance level of $p \leq 0.05$. Of the total samples analyzed, *Campylobacter* spp. were isolated in 86 (79.6 %), corresponding to 93 % *C. jejuni* and 7 % *C. coli*. Contamination according to origin ($p<0.05$): Cochabamba 96.4 %, La Paz 87.5 %, Santa Cruz 57.8 %. By type of market, the samples from Cochabamba showed no significant differences between popular markets (95.7 %) and supermarkets (100 %) ($p>0.05$). However, the samples from Santa Cruz showed a significant difference ($p<0.05$), with popular markets at 73.5 % and supermarkets at 9.1 %. According to the type of outlet, refrigerated and non-refrigerated samples from Cochabamba and La Paz ($p>0.05$) contamination did not present a significant difference that was higher than 66.7 %, however, in Santa Cruz refrigerated samples 42.8 % and 82.4 % in non-refrigerated samples at the time of sale ($p<0.05$). The observed percentages indicate that the conditions of sale and conservation are factors that influence food contamination and multiplication of the agent. These data are a significant contribution and a baseline for decision-making by the country's regulatory entities, as well as for recommending the control of factors that condition contamination and growth of this agent in food, as part of food safety

Resumen

Campylobacter spp. se considera un agente zoonotico y de distribución mundial, siendo uno de los principales reservorios las aves de corral, el objetivo de ésta investigación fue la detección de éste agente en carne cruda de pollo tomando en cuenta: la procedencia, modo de expendio y tipo de mercado, en la ciudad de La Paz. Para la detección del microorganismo se tomaron 108 muestras, aplicando el método analítico ISO 10272-1:2017 Parte 1: Método de detección. La investigación fue observacional transversal, para análisis estadístico se utilizó Microsoft Excel 2010 e InfoStat (2014). Los datos por factor se

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analizaron con la prueba de χ^2, con nivel de significancia de $p \leq 0.05$. Del total de muestras analizadas se aisló *Campylobacter* spp. en 86 (79.6 %), que corresponden a 93 % *C. jejuni* y 7 % de *C. coli* del total. La contaminación según procedencia ($p<0.05$): Cochabamba 96.4 %, La Paz 87.5 %, Santa Cruz 57.8 %. Por tipo de mercado, las muestras de Cochabamba no presentaron diferencias significativas entre mercados populares (95.7 %) y supermercado (100 %) ($p>0.05$). Sin embargo, en las muestras de Santa Cruz existe diferencia significativa ($p<0.05$), mercado popular 73.5 % y en los supermercados 9.1 %. Según tipo de expendio, las muestras refrigeradas y no refrigeradas procedentes de Cochabamba y La Paz ($p>0.05$) la contaminación no presentó diferencia significativa que fue superior al 66.7 %, sin embargo, en las muestras de Santa Cruz existe diferencia entre las refrigeradas 42.8 y 82.4 % en las no refrigeradas al momento de la venta ($p<0.05$). Por los porcentajes observados, se señala que las condiciones de venta y conservación son factores que influyen en la contaminación del alimento y multiplicación del agente, estos datos son un aporte significativo y como línea de base, para la toma de decisiones por parte de entidades reguladoras del país, por otra parte, para recomendar el control de los factores que condicionen la contaminación y crecimiento de éste agente en alimentos, como parte de la inocuidad alimentaria

Introduction

Campylobacteriosis is a zoonosis of worldwide distribution, caused by bacteria of the genus *Campylobacter*, the most frequent being *C. jejuni* and *C. coli*, characterized by causing invasive diarrhea, with abdominal pain, fever and general malaise. The main reservoirs are poultry, pigs and cattle. This pathogen is transmitted to humans by ingestion of raw or poorly cooked, contaminated food, mainly affecting children, the elderly and immunocompromised people[1]. It is also associated with the increase in the consumption of chicken meat, due to its low cost per kilo, compared to meat from other animal species in our environment (pork and beef). In Bolivia, its per capita consumption demand is 44 kg[2].

Currently, there are 66 species and 16 subspecies of *Campylobacter* recognized, most of which are associated with diseases in humans and animals[3]. Only 4 species are pathogenic to humans, the most frequent being *C. jejuni* and *C. coli*, and less frequently *C. fetus* and *C. upsaliensis*[1-4].

The *Campylobacter* genus is characterized by being Gram-negative, S-shaped bacilli, 0.2-0.8 µm wide and 0.5-5 µm long, with generally monotric polar flagella, they have a characteristic mobility, like that of a corkscrew or corkscrew, however, small thermophilic campylobacters have a rapid movement, are microaerophilic, therefore they need small amounts of oxygen (5 %) and capnophilic, that is, they need high amounts of carbon dioxide (10 %) and 85 % nitrogen[5]. Their metabolism is characterized by not oxidizing and not fermenting carbohydrates since they obtain their energy from amino acids and the 4 to 6 carbon intermediates of the Krebs cycle[6].

In the period from 2005 to 2013, *Campylobacter* was the most frequently reported gastrointestinal bacterial pathogen in humans in the European Union (EU). The number of confirmed cases of campylobacteriosis was 214779, with an EU notification rate of 64.8 per 100000 inhabitants, which was at the same level as in 2012, but the severity in terms of reported case fatality was low (0.05 %)[7]. Most recent data from 2021 from the European Centre for Disease Preven-

tion and Control (ECDC) shows that gastrointestinal campylobacteriosis was the most reported. That year, 30 countries that are part of the EU and the European Economic Area (EEA) reported 129690 confirmed cases, the overall notification rate in the EU/EEA was 44.5 cases per 100000 inhabitants. This decline in the notification rate occurred since 2020 due to the pandemic caused by the SARS CoV-2 virus[8].

In Bolivia, in 2009, a study was published on the monitoring of antibacterial resistance of *Campylobacter* spp. in four hospitals in La Paz. 370 stool samples were processed: 44 samples (12 %) were positive for *Campylobacter* spp., 28 were *C. jejuni*, 13 were *C. Coli* and 3 were *C. lari*[9].

In Bolivia, there is no published data on the presence of *Campylobacter* in raw chicken meat (RCM). There is a lack of work on the subject, and food microbiology laboratories (FML) have not yet detected this pathogen in food, which is why regulatory agencies do not take preventive measures to avoid food contamination, which becomes a vehicle for the transmission of diseases.

This research work aimed to evaluate the presence of *Campylobacter* in RCM at points of sale in the city of La Paz to obtain data on contamination factors such as: type of market, type of sale and origin that contribute to establishing a baseline for the prevention and control of foods susceptible to contamination with this bacterial agent.

Materials and methods

The work was carried out in the city of La Paz-Bolivia, which is divided into 9 macrodistricts, 2 rural and 7 urban, part of the Pedro Domingo Murillo province of the department of La Paz, located in the geographical position of South 16º 30' 0" (latitude) and West 68º 09' 0" (longitude). The city has a mountain climate and an average annual temperature of 14° C, with an average rainfall of 512 mm[10].

A cross-sectional observational study was carried out, by observing and recording events without intervening in their natural course, the measurement in relation to time was unique[11].

The samples were collected and processed from June 2018 to February 2019, and were processed at the FML of the Institute of Diagnostic Laboratory Services and Health Research (SELADIS) dependent on the Major University of San Andres. 108 whole RCM samples were taken in a non-probabilistic manner for convenience due to inclusion criteria in the sampling of 6 macrodistricts of the city of La Paz, taking into account the following study factors: i) origin (Cochabamba, Santa Cruz and La Paz), ii) type of market (popular and supermarket) and iii) mode of sale (refrigerated and non-refrigerated).

For the detection of *Campylobacter* spp. in RCM matrix, the Standard (ISO 10272-1:2017)[12] "*Microbiology of the food chain - Horizontal method for the detection and enumeration of Campylobacter* spp. *Part 1: Detection method*" was used (Method verified in SELADIS FML). The method applied consists of 4 stages which were: i) selective enrichment with a 1/10 dilution of the homogenized sample with Preston broth, and incubation in microaerophilia at 41.5º C for 24±2 h. ii) sowing of 10 µL on the surface by exhaustion in Modified charcoal cefoperazone deoxycholate agar (mCCD), and incubation in microaerophilia at 41.5º C for 44±4 h. iii) confirmation of characteristic colony-forming units (CFU), selection of suspected *Campylobacter* colonies for confirmation by oxidase test, morphology, motility and seeding of each of the selected CFU on 2 horse blood agar plates. To promote the development of the agent, one

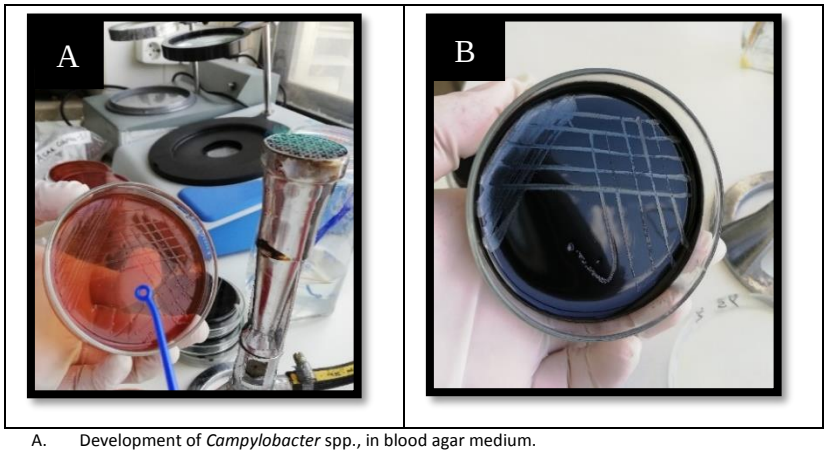
plate was incubated in microaerophilia at 41.5º C for 48 h and the other was incubated at 25º C for 48 h in aerobiosis. iv) differentiation of *C. jejuni* and *C. coli* species by biochemical tests of catalase activity, indoxyl acetate hydrolysis and hippurate hydrolysis.

For data analysis, the statistical package Microsoft Excel 2010 and InfoStat, version 2014, were used, and the results were expressed in percentages, contingency tables, frequency distribution and chi-square test (χ^2).

Results

Of 108 RCM samples analyzed, 79.6 % (86) showed the presence of *Campylobacter* spp. (Figure 1), this being one of the first reports of the presence of the microorganism, object of study, in the city of La Paz. Analyzing the 86 positive samples, 93 % (80) were identified as *C. jejuni* and 7 % (6) as *C. coli* (Figure 2) (Table 1).

Figure 1 Growth of *Campylobacter* spp. on blood agar and mCCDA



A. Development of *Campylobacter* spp., in blood agar medium.
B. Development of *Campylobacter* spp., in Modified charcoal cefoperazone deoxycholate agar (mCCD) medium.

Table 1 Presence of Campylobacter in raw chicken meat at points of sale in the city of La Paz - Bolivia

Macrodistrict	Number of samples*	*Campylobacter* spp.		Presence (%)	
		Presence (%)	Absence (%)		
Cotahuma	18	16 (14.8)	2 (1.85)	16 (18.6)	0
Periférica	18	14 (13.0)	4 (3.7)	13 (15.0)	1 (1.2)
Max Paredes	18	16 (14.8)	2 (1.85)	15 (17.4)	1 (1.2)
Zona Central	18	13 (12.0)	5 (4.6)	10 (11.6)	3 (3.4)
San Antonio	18	15 (13.9)	3 (2.8)	14 (16.5)	1 (1.2)
Zona Sur	18	12 (11.1)	6 (5.6)	12 (13.9)	0
Total	108	86 (79.6)**	22 (20.4)	80 (93.0)**	6 (7.0)**

*Total number of samples per macrodistrict is 18 raw chicken meats
** Of 86 samples showing the presence of Campylobacter in raw chicken meat, 80 are *C. jejuni* and 6 are *C. coli*

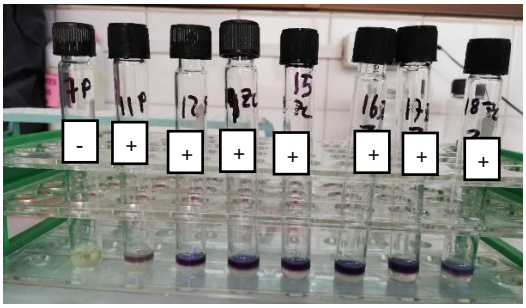
Table 2 Presence of *Campylobacter* spp. in raw chicken meat by origin

Origin	Number of samples	*Campylobacter* spp.	
		Presence (%)	Absence (%)
Cochabamba*	55 (100 %)	53 (96.4)	2 (3.6)
Santa Cruz*	45 (100 %)	26 (57.8)	19 (42.2)
La Paz*	8 (100 %)	7 (87.5)	1 (12.5)
Totales	108	86	22

* χ^2 (2) = 23.05, p<0.05.

By origin (Table 2), significant statistical differences were evident (χ^2 (2) = 23.05, p<0.05), indicating that the presence of *Campylobacter* spp. in RCM is dependent on its origin, at a level of 95 % reliability. The samples from Cochabamba and La Paz had a contamination percentage of 96.4 and 87.5 % respectively, figures higher than the 57.8 % observed in Santa Cruz.

Figure 2 Hippurate Hydrolysis Test for Differentiation between *C. jejuni* and *C. coli*



Negative (-), *C. coli;* Positive (+) *C. jejuni*

Regarding the type of market (Table 3), the RCM from Cochabamba did not show statistically significant differences (χ^2 (1) = 0.35, p>0.05) in relation to the presence of *Campylobacter* spp., regardless of whether it was sold in a popular market or supermarket. In contrast, the samples from Santa Cruz had a statistically significant difference (χ^2 (1) = 14.5, p<0.05) depending on the type of market. It was observed that the presence of this microorganism was significantly lower in supermarkets (1 positive sample out of 11 analyzed), compared to popular markets (25 positive samples out of 34 analyzed).

Analyzing the type of sale (Table 4), the RCM that come from Cochabamba and La Paz, there were no statistical differences (p>0.05), therefore, the presence of *Campylobacter* in the samples under study, is independent of whether the samples were refrigerated (100 % contamination) or not refrigerated (93.3 % contamination).

Table 3 Presence of *Campylobacter* spp. in raw chicken meat by market type

Study factor	Type of market	Number of samples	*Campylobacter* spp.	
Origin			Presence (%)	Absence (%)
Cochabamba	Mercado popular*	47 (100 %)	45 (95.7)	2 (95.7)
	Supermercado*	8 (100 %)	8 (100)	0 (0.0)
Santa Cruz	Mercado popular**	34 (100 %)	25 (73.5)	9 (26.5)
	Supermercado**	11 (100 %)	1 (9.1)	10 (90.9)
Total		100	79	21

* χ^2 (1) = 0.35, p>0.05, ** χ^2 (1) = 14.5, p<0.05.

Table 4 Presence of *Campylobacter* spp. in raw chicken meat by type of sale

Study factor	Type of market	Number of samples	*Campylobacter* spp.	
Origin			Presence (%)	Absence (%)
Cochabamba	No refrigerado*	30 (100 %)	28 (93.3)	2 (6.7)
	Refrigerado*	25 (100 %)	25 (100)	0 (0.0)
La Paz	No refrigerado**	5 (100 %)	5 (100)	0 (0.0)
	Refrigerado**	3 (100 %)	2 (66.7)	1 (33.3)
Santa Cruz	No refrigerado***	17 (100 %)	14 (82.4)	3 (17.6)
	Refrigerado***	28 (100 %)	12 (42.8)	16 (57.2)
Total		100	79	21

* χ^2 (1) = 1.73, p>0.05. ** χ^2 (1) = 1.90, p>0.05. *** χ^2 (1) = 16.79, p<0.05.

In contrast, in the samples from Santa Cruz there is a significant statistical difference (χ^2 (1) = 16.79, $p<0.05$), observing that the contamination of the RCM is dependent on the type of sale, with a percentage of 82.4 % contamination in non-refrigerated samples, compared to refrigerated samples which is 42.8 %.

Discussion

The detection of *Campylobacter* spp. in RCM was high 79.6 % (Table 1), results that agree with the data reported in Latin America, such as Mexico (89 %)[13], Peru (97%) in markets in the province of Lima[14], Chile (63.3 %)[15] and Ecuador (71 %)[16].

RCM contamination with *C. jejuni* was 93 % compared to *C. coli* 7 %, several works reported a lower percentage of *C. jejuni* in RCM, thus, in a work carried out in Chile in 2017 it was reported (63.3 %)[15]. In South America, data has been reported for more than 15 years, in Argentina (87.8 %)[17], and Brazil (68.8 %)[18].

C. jejuni is found in the digestive tract of poultry, for this reason, a high number of chicken carcasses are contaminated[15]. The presence of the microorganism is associated as one of the main causes of enteric zoonotic infections in the world. The World Health Organization (WHO) considers it as the first etiological agent of diarrhea in humans in developed countries, and the second or third in developing countries, such as Latin America[1].

Since 2008, it has been reported that campylobacteriosis is one of the most prevalent zoonoses in developed countries, due to the contamination of food of animal origin, mainly in poultry products[19]. The report by the European Food Safety Authority and the ECDC, noted the results of monitoring zoonoses in 36 EU countries, establishing that campylobacteriosis is in first place, salmonellosis in second and *Escherichia coli*, producer of Shiga toxin, in third place[20]. *Campylobacter* is prevalent in poultry intended for human consumption and generally does not cause disease in them[1]. Due to this characteristic, campylobacteriosis is not detected in poultry farms, so contamination can start in the production area, continue to the processing plants (slaughterhouses) and reach the points of sale[21].

In Bolivia, the Departmental Service of Animal Health and Food Safety (SENASAG) only has a National Program for the Eradication of Salmonellosis in chickens[22]. Currently, there is no regulation or control and surveillance program for *Campylobacter*, despite the increase in Foodborne Illnesses (ETAs) with this agent worldwide[1]. It is considered important to implement a surveillance program for this agent throughout the food chain, from primary production to the consumer.

The WHO mentions that *Campylobacter* spp. is included among the 4 main causes of diarrheal diseases, with children under 5 years of age being the most affected, representing one third of foodborne diseases[23].

To avoid cross contamination, it is important to implement hygiene and handling measures for proper handling of chicken meat when cooking, since it is a vehicle for the transmission of diseases caused by *Campylobacter*[1].

When evaluating Table 2, it was evident that the origin of the RCM is related to the percentage of presence of *Campylobacter* spp., with the one from Cochabamba having a presence of 96.4 %. This data is alarming, because the 2 producing companies, according to their advertising, had ISO 9001:2015 certification, possibly their certification is not oriented to the safety of the product. In the meat samples from

La Paz, the percentage of contamination with this agent was 87.5 %, and the suppliers were small and medium-sized producers from the north of La Paz. In comparison, the samples from Santa Cruz presented 57.8 % contamination by *Campylobacter* spp., which could be attributed to the fact that this poultry company is certified with ISO 9001 and 22000, which certifies food safety, implemented to ensure the quality of its product throughout its production chain.

Analyzing Table 3, the relationship between the type of market and the presence of *Campylobacter* spp. in RCM was evaluated. As previously discussed, the origin of the samples had a statistical relationship with the presence of the microorganism, so it was decided to analyze the type of market taking into account the origin, with the objective of evaluating individually if the type of market is related to the type of handling, and becomes relevant in the contamination of RCM. The analysis took into account only the meat from Cochabamba and Santa Cruz, because the samples from La Paz are distributed only in popular markets. The samples from Cochabamba showed that the contamination of the RCM is independent of the type of market, which could indicate that the samples could have been contaminated in one of the stages of the production chain[21]. This analysis contrasts with the results obtained in the samples from Santa Cruz, the type of market significantly influences ($p<0.05$) the contamination of the samples obtained, with the meat sold in popular markets having a significantly higher contamination (73.5 %) compared to that in supermarkets (9.1 %). This result may be due to the fact that in the on-site observation, there is a difference in the way sales and handling areas are organized. Supermarkets only sell whole packaged chicken and arranged in exclusive spaces by brand and in refrigeration. In popular markets the chicken was arranged together without differentiating the brand and without individual packaging, transported in plastic baskets and the handling was carried out without gloves, using the same knife to divide the chicken pieces and giblets, conditions that lead to cross contamination, affecting the safety of the food at the time of sale.

When analyzing the impact that the type of chicken meat sales has on the presence of *Campylobacter* spp. it was decided to evaluate this relationship of variables individually for each origin. In the samples from Cochabamba and La Paz, the statistical analysis revealed that the type of sales, whether refrigerated or not, did not have a significant impact on RCM contamination. This finding suggests that chicken contamination could occur before the sale of this food product, that is, at any point in the production chain.

In contrast, when examining the statistical relationship between the type of sales and the presence of *Campylobacter* spp. in the samples from Santa Cruz, it was clearly observed that refrigeration did have a statistically significant relationship. This result indicates that contamination is lower when the cold chain is maintained during distribution, while the presence of *Campylobacter* is favoured in places of sale where the meat is not refrigerated. This data is consistent taking into account that for the conservation of chicken meat it is essential to maintain the cold chain at less than 7° C, in order to avoid the multiplication of microorganisms due to the effect of temperature.

These findings are consistent with the need to maintain the cold chain below 7° C to prevent the multiplication of microorganisms due to temperature, which is crucial for the proper preservation of chicken meat.

The stalls in popular markets, where there is a greater attendance of consumers, do not meet the conditions for the sale of this product, since they do not maintain

the cold chain. It was observed that the meat is exposed on counters and for several hours at room temperature, which conditions the reduction of the product's shelf life. Preservation by refrigeration is an ideal method for preserving food that consists of keeping it at a temperature of 4-6° C in order to reduce bacterial activity.

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Conflicts of interest

This article was prepared and reviewed with the participation of the authors, who declare that there is no conflict of interest that could jeopardize the validity of the results presented**.**

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Ethical considerations

This study was carried out on raw chicken meat intended for human consumption, so the analysis of these samples does not lead to an ethical or moral conflict.

Authors' contribution to the article

Marcelina Condori Ticona, development of the research, development of the microbiological technique, sample collection, writing, review and editing and interpretation of the results, preparation of the original draft, preparation of the article. *María del Pilar Donado Godoy*, review of the article. *Angélica María Espada Silva*, management and administration of the project, search for information, training and development of microbiological techniques, supervision, evaluation and interpretation of results and final review of the document.

Research limitations

There were no limitations in the research.

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