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Pathogens of zoonotic interest in chicken meat for sale in retail stores in Mexico

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ABSTRACT

Objective: Determine the presence of zoonotic pathogens in chicken meat sold in retail centers in five cities of the Mexican Republic.

Design/methodology/approach: 153 samples of raw chicken meat were analyzed. All samples were analyzed using methods approved by the AOAC and the US regulatory agencies, isolation that what promised, slipped and subsequently PCR analysis was performed for *Campylobacter* spp., *Salmonella* spp., *E. coli* and *Listeria* spp.

Results: *Campylobacter* spp. it was found in 31% of the samples and *Salmonella* spp. in 1.31% of the total samples analyzed. *Campylobacter* spp. it has a higher prevalence in Tlalnepantla Estado de Mexico (74%), Puebla (33.33%) and Guadalajara Jal. (25.58%). *Salmonella* spp. it has a higher prevalence in Tlalnepantla Estado de Mexico (3,7%) and Guadalajara Jal. (4,65%) sites.

Limitations: This study describes the prevalence of *Campylobacter* and *Salmonella* in chicken meat for sale in Mexico, however, more studies are needed to determine exactly the origin of these bacteria scale.

Findings/conclusions: According to the results obtained in this work, it can be concluded that there is contamination of the chicken meat with the bacterium *Campylobacter* spp. in a higher proportion, unlike *Salmonella* spp. This may be due to possible errors in the handling in the different areas by which the bird is handled from the farm to the commercialization.

Keywords: Chicken meat, Zoonoses, Broilers, Bacterial contamination.

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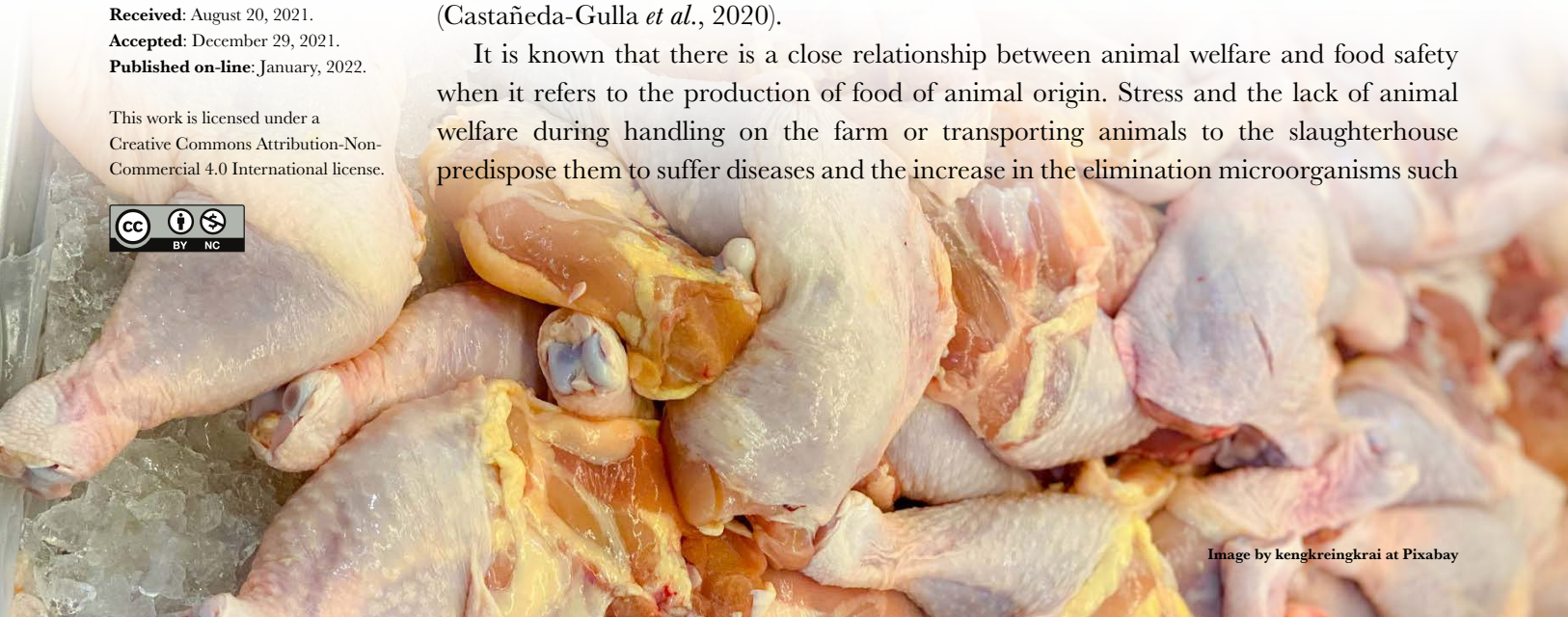
INTRODUCTION

Chicken meat is a food of great nutritional importance in the diet of humans, however, its consumption may represent a health risk, since in order to satisfy the demand of the population, it is intensively produced and it is processed in an industrial way and as a consequence there is greater stress for animals causing diseases that can affect human being (Castañeda-Gulla *et al.*, 2020).

It is known that there is a close relationship between animal welfare and food safety when it refers to the production of food of animal origin. Stress and the lack of animal welfare during handling on the farm or transporting animals to the slaughterhouse predispose them to suffer diseases and the increase in the elimination microorganisms such



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as *Campylobacter*, *Salmonella* and *Echerichia coli* through feces, causing the contamination of meat with these pathogens and their toxins putting the health of consumers at risk (FAO/WHO, 2009; Rostagno, 2009; López *et al.*, 2016; Alpigiani *et al.*, 2017; EFSA, 2019). Made additionally worrisome because of the exposure to stress conditions during the slaughter process can induce bacterial adaptation as a result of changes in the genetic expression of these pathogens (Duqué *et al.*, 2021).

In the case of *Campylobacter* spp. it represents up to 70% of zoonosis cases in Europe, followed by *Salmonella* spp. both are related to products derived from the poultry sector (EFSA / ECDC, 2018) and have been isolated from live birds and in meat ready for sale (Berndtson *et al.*, 1996; Willis *et al.*, 1997; FAO-WHO, 2009). *Campylobacter* spp. is one of the four main global causes of diarrheal disease and is considered the most common bacterial cause of gastroenteritis in the world. Complications such as hepatitis, pancreatitis and abortions have also been observed, with varying degrees of frequency. Post-infection complications include reactive arthritis and neurological disorders such as Guillain-Barré syndrome (WHO, 2021).

Salmonella is transmitted through food and according to the European Union (Regulation (EC) No. 2160/2003) chicken meat is one of the main sources of transmission to humans causing salmonellosis and typhoid fever. It is reported that more than 1,000,000 non-typhoid *Salmonella* infections annually (EFSA, 2015; CDC, 2018), while in Mexico the number reaches around 70,000 cases of this disease each year (DGE, 2017).

Another microorganism of importance for public health is *E. coli*, which is found in 90% of the feces of birds and is recognized as an indicator of fecal contamination. In recent years it has been the cause of outbreaks with negative impact worldwide (FAO, 2021).

In Mexico, poultry production is very important, representing 0.81% of the national Gross Domestic Product (GDP), 36.65% of agricultural GDP and 63.3% of national livestock production (UNA 2019), most of the production units are of the intensive type and about 3 million 377 tons of chicken meat are produced (FAOSTAT, 2021), with a per capita consumption of 29 kg (UNA, 2019).

Mexico has a population of 126,014,024 inhabitants, of which 7% correspond to children under four years old, 8.5% to children between 5 and 9 years old, ages where children are most susceptible to contracting gastrointestinal diseases that can cause death and these are due to the consumption of contaminated food. But also, between 10 and 29 years of age it takes importance since they represent 15% of the total population (INEGI, 2021) and are the most common hosts of this type of pathogens. This being a reason to consider this as a food safety problem. Furthermore, in Mexico the impact on health is unknown and no data was found on campylobacteriosis in animals destined for human consumption.

For all the above, the objective of this study was to preliminarily detect the presence of *Campylobacter* spp., *Salmonella* spp., *E. coli* and *Listeria* spp. in chicken meat for sale in retail centers in five main cities of the Mexican Republic.

MATERIALS AND METHODS

In the months of December 2020 and January 2021, 153 samples of raw chicken meat were collected from different points of sale of the most important retail centers in

5 cities of the Mexican Republic (Tijuana BC; Guadalajara, Jal; Tlalnepantla Estado de México; Puebla and Queretaro), selected according to the importance they have for the production of broilers. The samples were collected in a period of 3 hours and transported in refrigerators with ice for transport and stored in refrigeration at 3 °C until analysis.

Sample processing and analysis

All samples were analyzed using methods approved by the AOAC and US regulatory agencies.

The analytes and methods were:

Campylobacter: first the isolation of *Campylobacter* spp. was carried out, carrying out the initial detection (presumably positive) in 48 hours, to later carry out the isolation and confirmation in a total of 4 days. Using 1625±32.5 mL of Buffer Peptone Water (BPW) which was added to 325±32.5 g of raw chicken meat. To disperse lumps, the samples were thoroughly mixed by brief manual massage using a bag (no more than 10 seconds). After mixing, 30 mL of double concentration bloodless Bolton enrichment broth was added, 30 mL of the sample was placed in a bag, mixed and shaken by hand several times. The samples were incubated for 48±2 hours at 42±1 °C under microaerobic conditions by placing 2-3 CampyGen containers (Fisher Scientific, Leicestershire, UK) inside an anaerobic flask. After 48±2 hours of incubation, 3-5 mL of enriched broth were taken for PCR analysis. The analysis was performed in a multiplex PCR for identification of *Campylobacter jejuni* and *Campylobacter coli*. This standard operating procedure is based on USDA MLG 41.05 (2021) and uses Bolton double concentration bloodless enrichment broth for enrichment and multiplex PCR for identification of *C. jejuni* and *C. coli* from broth and agar plates (isolation). Identification was carried out with multiplex PCR assays and confirmation was carried out by colony isolation on agar plates and latex agglutination test.

For the case of *E. coli* O157, STEC and *Salmonella*: PCR technology was used to amplify unique DNA sequences present in *E. coli* O157, other Shiga toxin-producing *E. coli* (STEC) and *Salmonella* that cause disease in humans. Subsequently, a 75 g sample was directly enriched with 150 mL of M1-GN broth using filter bags and incubated at 42 °C for a minimum of 12 hours (maximum 24 hours) and analyzed with the AOAC PTM 100701 "IEH methodology. *E. coli* O157, producer of Stx *E. coli* (STEC) with Intimin & Salmonella Test System", a PCR-based method that has genetic targets for *Salmonella*, *E. coli* O157 and STEC O26, O45, O103, O111, O121 and O145.

Listeria monocytogenes: the PCR method was used to amplify unique DNA sequences. present in *Listeria monocytogenes* and *Listeria* spp. that cause diseases in humans. A 75 g sample was placed in 150 mL of M1-GP medium using filter bags and incubated at 35 °C for a minimum of 21 h (maximum 48 h). After enrichment of the sample, the bacterial DNA is released from the organisms in special buffer by a lysis procedure. Two unique specific bacterial DNA fragments of *Listeria* spp. and two unique specific fragments of *Listeria monocytogenes*, not present in other bacteria, are targeted, and amplified using Taq DNA polymerase and nucleotides. After PCR amplification, the products were separated by agarose gel electrophoresis and visualized by a UV transilluminator after being stained with ethidium bromide (EtBr).

Statistical analysis

The experimental design was completely randomized, and the results obtained were analyzed by calculating the prevalence through descriptive statistics using the SPSS 18 package and the comparison between sampled cities was carried out by using a General Linear Model (GLM) and for the comparison for means, the Tukey test was used, using the statistical package SAS 9 (2002).

RESULTS AND DISCUSSION

E. coli and *Listeria* spp. were not detected in the raw chicken meat samples analyzed in this work. But the presence of *Campylobacter* spp. was detected in 31% of the total samples analyzed, showing a significant difference ($p<0.05$) (Figure 1), the percentage of positive samples for this bacterium being higher in Tlalnepantla State of Mexico 74%, Puebla with 33.33% and Guadalajara Jal. 25.58% compared to the analyzed samples from Tijuana BC 18.5% and Queretaro 10.52% (Table 1).

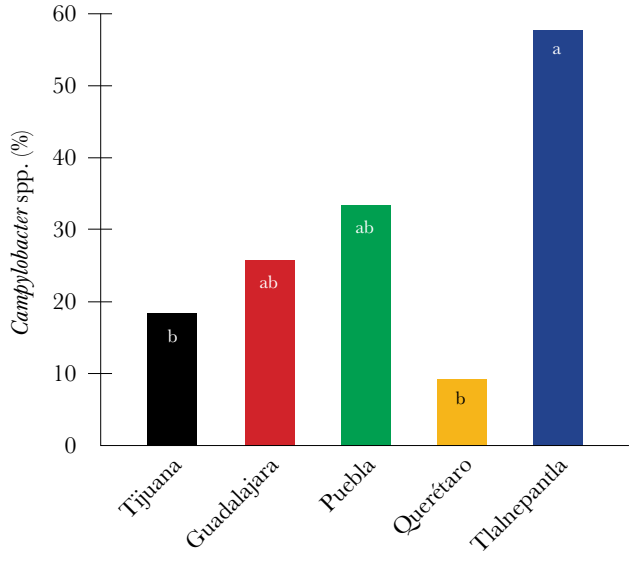

Figure 1. *Campylobacter* spp. in chicken meat for sale in retail stores in México. Literals (a, b) represents significant difference ($p<0.05$).

Table 1. Percentage of incidence of pathogens of zoonotic interest in chicken meat for sale in retail establishments in Mexico.

City	*Salmonella* spp	*E. Coli*	*Campylobacter* spp.	*Listeria* spp.
Tijuana	0/27	0/27	5/27	0/27
Tlanelpantla	1/27	0/27	20/27	0/27
Guadalajara	2/43	0/43	11/43	0/43
Puebla	0/27	0/27	9/27	0/27
Queretaro	0/19	0/19	2/19	0/19
Total	2/153	0/123	47/153	0/153
Incidence rate (%)	1.3	0	31	0

In the case of *Salmonella* spp. it was found in 1.3% of the total analized chicken meat samples, with no significant difference ($p>0.05$) between Tlalnepantla Estado de Mexico (3.7%) and Guadalajara (24.65%), but it did showed a significant difference ($p<0.05$) between these two cities and the rest of the sampled cities (Puebla, Tlalnepantla, Queretaro) in which it was not detected.

In many places worldwide, *Campylobacter* spp. has been detected in chicken meat and Mexico is no exception, and this bacterium is considered a frequent cause of gastrointestinal disease, the higher the rate of contamination of the animals, the greater the risk of contamination of the products obtained. after its process in the slaughterhouse; the main source of contamination being the content of the intestinal tract, where it can colonize in a high number of 1010 colony forming units (CFU) per gram of infected intestine, the main site of colonization being the cecum, where *Campylobacter* spp. it is found in the mucous layer of epithelial cells (Hernández-Cortez *et al.*, 2013). In addition, the bacteria can be present on the skin or feathers; thus, animals from uninfected flocks become contaminated during slaughter, especially in the plucking, evisceration and refrigeration process (Mead *et al.*, 1995; Keener *et al.*, 2004; Cervantes-García, 2020).

In this work, 31% of the raw meat samples appeared contaminated with *Campylobacter* spp. a value that is similar to that reported by EFSA (2015) for raw chicken meat samples (31.4%) in the European Union. However, the value found in this work is below that reported in raw meat by Zhao *et al.* (2001) (70%) in New York, Chrystal *et al.* (2008) (44.8%) in New Zealand, Zaidi *et al.* (2012) Mexico (58.3%), Zumbado-Gutierrez *et al.* (2014) (40%) in Costa Rica and above that found by Lucas *et al.* (2013) in Peru 16.7%, Di Giannatale *et al.* (2019) (17.38%) in Italy and by Thomas *et al.* (2020) who at carrying out a bibliographic review in Africa found that the prevalence of *Campylobacter* spp. in chicken meat was 21% of a total of 2973 samples from different African regions.

The presence of *Campylobacter* spp. in the samples of chicken meat sampled in this work, it could be due to the fact that it is a bacterium that lives in the intestine of birds and the stress is due to the lack of animal welfare during handling on the farm, transport and/or the process. of the slaughter contributes to the animals eliminating a greater amount of this micro-organism and its toxins through the feces, causing the contamination of the work equipment, the surfaces, the process water and the air, and with this there is a greater dissemination of the bacteria with the consequent contamination of the meat (Iannetti *et al.*, 2020). Therefore, greater interest should be placed on animal welfare on the farm, food safety practices and operations in processing plants (Dogan *et al.*, 2019).

According to the percentage of contaminated samples found in this work and considering that in Mexico there is a per capita consumption of 29 kg, and the annual production amounts to 3 million 377 tons of chicken meat (FAOSTAT, 2021), the data found is alarming, since it would represent a contamination of approximately 1,046,840 tons of raw chicken meat per year, the risk being high for a population between children and adults. If taking into account that a contaminated bird carcass can carry between 100 and 100,000 *Campylobacter* cells and that only 500 cells are required to cause infection (Hernández-Cortez *et al.*, 2013), then there would be a number of exposed inhabitants of approximately 36,098,000 per year, corresponding to 272,537 daily exposures nationwide.

This could be greater than the 845,024 cases per year confirmed at the laboratory level in the United States of America (Scallan *et al.*, 2011; Dogan *et al.*, 2020). This is relevant since the WHO (2021) reports that 1 in 10 people in the world suffer from diarrheal diseases and in the case of children under 5 years of age affected reach up to 220 million, where this disease can be fatal, being *Campylobacter* spp. one of the four most prevalent pathogens in food worldwide.

On the other hand, the presence of *Salmonella* spp. was identified in 2 of the samples of a total of 153 that were collected for the work, representing 1.3% of positive samples in raw chicken meat. The prevalence found in this work is below that reported by Van *et al.* (2007) Vietnam 53.3%, Pointon *et al.* (2008) Australia 43.3% and Adeyanju *et al.* (2014) in Nigeria 33%. The prevalence of *Salmonella* spp. can vary depending on the geographic region, the zootechnical and manufacturing practices, distribution and the biosecurity programs for the control of pathogens. In this work, during the sampling period, the prevalence is low, however, it would be important to carry out this same analysis in times of heat and high rainfall where weather conditions could influence a greater presence of this bacterium (Akil *et al.*, 2014).

CONCLUSIONS

According to the results obtained in this work, it can be concluded that there is contamination of the chicken meat with the bacterium *Campylobacter* spp. in a higher proportion, unlike *Salmonella* spp. This may be due to possible errors in the handling in the different areas by which the bird is handled from the farm to the commercialization. This work serves as preliminary information and determines the need to improve the handling of the animals from the farm to the slaughterhouse in order to avoid stress and contamination with pathogens in chicken meat and thus avoid the risk to the health of the chicken's consumers. It is important that health authorities implement prevention programs for these diseases through the regulation and control of the production, distribution and marketing of poultry products and their derivatives.

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