



Presence of *Campylobacter* spp. in Whole Chickens and Viscera Marketed in the Municipality Girardot Aragua State, Venezuela

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Abstract: Consumers of food products of animal origin, require compliance with good manufacturing practices, to ensure their safety. The presence of pathogens able to produce food (ETAs) transmitted diseases, justify the urgent need to determine the presence of *Campylobacter* spp. This research descriptive transversal, aimed at detecting the presence of *Campylobacter* spp in broilers packed whole and viscera, marketed in the municipality Girardot of the State Aragua, Venezuela, where they were collected through a non-probability sampling weekly four chickens of three production batches, during June 2013, a total of 48 chickens and 48 groups of viscera. They were assessed by rapid plate test; finding *Campylobacter* spp in lot 1 100% for broilers and viscera, in Lot 2 68.75% in one chickens and 50% in viscera and Lot 3 75% and 56.5% in chicken and viscera; averaging 81.25% for whole chickens and 68.50% for viscera. The number of colony forming units (CFU) than the infective dose for individual's ≥ 500 CFU, was obtained in 43.75% of the chickens and viscera 25% lot 1, 12.5% of broilers and viscera lot 2 and 6.25% of chickens and viscera lot 3. In determining the degree of correlation between the UFC in chickens and viscera an association between these variables ($P < 0.005$) was observed.

Keywords: *Campylobacter* spp, Whole Chickens, Offal, Prevalence, Safety

1. Introduction

The process of industrialization of the poultry sector has achieved a high degree of automation, however, such progress does not translate into an improvement in the quality of the meat, rather, they contribute to increase the microbial load of the poultry carcasses gaining importance today *Campylobacter* spp, among other microorganisms involved in food-(ETAs) transmitted diseases [1]. Infections among species of the family or *Campylobacteraceae* have campylobacteriosis *Campylobacter enteritis*, considered the most important in public health, its main agents are *C. jejuni* and *C. coli* (can also cause systemic infections and complications after infection; Agents Guillain Barre GBS) the impact of public health campylobacteriosis is increasing [2].

The genus *Campylobacter*, is dated and comprising gram negative bacilli curved (gullwing), with polar flagellation,

microaerophilic, do not use sugar, but energy of amino acids, are thermotolerant species 42°C, zoonotic, birds are an important reservoir, are the causative agents of diarrhea in humans (first cause in industrialized countries and second or third cause in Latin America). They have been isolated 25 species and 9 subspecies [2].

Campylobacter spp, requires optimal growth conditions (5% O₂, CO₂ 3-15% and 85% N), mentioned three species of *thermophilic Campylobacter* causing significant health problems in humans (*C. jejuni*, *C. coli*, *C. laridis*) and outnumber cases of enteritis caused by *Salmonella* sp. and *Shiguellas* sp. [3]. According Seminar INFAL 2015 [4], following up on time pathogens *Campylobacter* spp, it was reported steadily increasing in England and Wales between 1997 and 2002 beating Rotavirus and Salmonella; even as [5], at the Ninth International Congress of Tropical Medicine and Health held in Sweden indicated that cases of

campylobacteriosis were more than doubled between 1988 and 2013, i.e. 3127 cases in 7499, an issue that has worsened since 1995. The center for Disease Control and Prevention (CDC) [6], in its surveillance program of *Campylobacter* spp said that for this year experienced an increase of 14% over the years 2006-2008; noting that for every case of campylobacteriosis reported, there are 30 undiagnosed cases

Domestic and wild animals serve as host to the bacteria, causing pollution 90% of chicken carcasses during processing ([7]. Studies conducted in Venezuela, specifically in the Aragua state by [8] reported *Campylobacter* spp in samples of whole, breasts, thighs and wings 75%, 95.83%, 83.33% and 70.83% respectively chickens, found in the first three samples indicated conditions ≥ 500 units colony forming (CFU) per ml. Other researchers [9], [10] found *Campylobacter* spp in a 70.83% in liver and 48.95% in chicken gizzards; likewise [11] in Chile reported that poultry liver 95.1% recorded in Brazil isolation and a percentage that goes from 13.5 to 78.7.

Campylobacteriosis is a zoonotic disease caused by eating food contaminated with bacteria of the genus *Campylobacter* as raw milk, seafood, poultry and other animals (cross-contamination), as well as in the untreated water; It occurs most often in children and young people, where it was reported 24.08 and 10.54% respectively in 2012 and in adults between 20 and 64 years and over reported 14.54 and 15.26. It is characterized by diarrhea, cramping, abdominal pain, fever, nausea and vomiting, some sequels neurological in which the syndrome Gillian Barre Syndrome (GBS) and Miller Fisher syndrome (MFS) [12].

The aim of the study was to analyze the prevalence of *Campylobacter* spp in whole chickens packed and viscera three lots traded in the municipality Girardot Aragua, Venezuela State through a quick test plate, and infer risk to which it is subjected consumer; also alert the population and institutions to exercise an active epidemiological surveillance in terms of ensuring public health.

2. Methodology

2.1. Taking the Sample

In this descriptive and cross-sectional research was acquired by a non-probability sampling four chickens in each of three lots, weekly reach outlets increased demand in the municipality Girardot Aragua state, Venezuela; in June 2013, to give a total sample of 48 samples of whole chickens and viscera; these were transported in ice coolers to the laboratory of Public Health of the Central University of Venezuela, Maracay Estado Aragua for a time did not exceed 30 minutes. Samples were collected and recorded in the collection protocol and processed under strict hygiene materials, equipment and surfaces used.

2.2. Procedures

Simple rapid test used in detecting the presence *Campylobacter* spp, which is a rapid, commercial, easy to

prepare, sensitive and useful test in processing large numbers of samples, was used following the indications for use [13], [14] as described below.

i. Rifampicin Additive

0.25 gg of rifampicin, diluted in 60-80 ml of alcohol, add distilled water to a final volume of 100ml.

ii. Hemin Additive

Mix 10ml of NaOH in 90 ml water, add 0.4 g of hemin continuously stir to homogenize the mixture.

Phosphate buffered water for dilutions

Buffered water for dilution: 1.25 ml of phosphate buffer stock solution was mixed into one liter of distilled water, sterilized by autoclaving at 121°C for 15 min. It was prepared and distributed in bottles of the required amount of dilution solution to wash the samples and viscera.

All reagents described are preserved under refrigeration.

2.3. Washing of Carcasses and Offal

Chicken or viscera within a sterile bag was introduced, it was added 500 ml of phosphate buffered solution, stirred manually about 2 min, for washing the sample.

2.4. Procedure for Conducting Rapid Test Plate Used for Whole Chicken and Viscera

(1) Nine (9) ml of distilled water to the vial (half Simplate) was added, homogenized and 0.025 ml was added 0.040 ml Rifampicin and Hemin. After washing 1ml of the sample, was added. The vial contents was placed in the center of the plate evenly distribute must endeavor all wells

(2) Seeded plates were placed one upon the other in the grid carrier plates and the jar microaerophilic, microaerophilic embedded envelope (oxid®), the tab holder on located on the side of the grid plates porta pitcher, was introduced into the jar, quickly covered and incubated in the oven at 42°C for 48 hours.

2.5. Interpretation of Results

All red little wells were counted, they are presumably positive for *Campylobacter* spp. Subsequently he placed in a UV lamp, were counted and the number of wells with fluorescence was recorded and subtracted the number of red wells, obtained in the previous step. The result started Simplate conversion table and the number of colony forming units (CFU) was obtained for that sample. The results descriptive statistical analysis and analysis matching underwent Kendal.

Table 1. Conversion table Simplate ® to colony forming units (CFU).

Population=wells positive	population=positive wells	Population=wells positive
1=2	29=70	57=190
2=4	30=74	58=196
3=6	31=76	59=202
4=8	32=80	60=208
5=10	33=84	61=216
6=12	34=86	62=224
7=14	35=90	63=232

Population=wells positive	population=positive wells	Population=wells positive
8=16	36=94	64=240
9=18	37=96	65=248
10=22	38=100	66=256
11=24	39=104	67=266
12=26	40=108	68=276
13=28	41=112	69=288
14=30	42=116	70=298
15=32	43=120	71=312
16=36	44=124	72=324
17=38	45=128	73=338
18=40	46=132	74=354
19=42	47=136	75=372
20=46	48=142	76=392
21=48	49=146	77=414
22=50	50=150	78=440
23=54	51=156	79=470
24=56	52=160	80=508
25=58	53=166	81=556
26=62	54=172	82=624
27=64	55=178	83=738
28=68	56=184	84=>738

3. Results

Bacteriological study showed the presence of *Campylobacter* spp, where rapid test detected a 68.75% in the 48 samples of whole chickens, 81.25% (39/48) of *Campylobacter* spp positivity and viscera (33/48). The results in percentage values of positivity in chickens and viscera, found in different batches of product analyzed, are presented discriminately, as shown:

3.1. Whole Chickens

In Table 2, the results of the 48 samples of whole chickens are indicated, the rapid test detected the presence Simplate® 100% (16/16) positive for *Campylobacter* spp, for lot 1; 68.75% (11/16) lot 2 and 75% (12/16) lot 3.

Table 2. Percentage Distribution of *Campylobacter* spp positivity in samples of whole chickens three lots traded in the municipality Girardot Aragua state, Venezuela.

% POSITIVITY		
LOT	No. of samples	Whole Chickens
1	16	100
2	16	68,75
3	16	75
TOTAL	48	81,25

A. The results are expressed as the percentage of positive rapid test samples.

3.2. Viscera

48 viscera of chickens tested positive lot 1 was 100% (16/16) *Campylobacter* spp, for lot 2, 50% (8/16) and 56.25% (9/16) Lot 3 (Table 3). Regarding the comparison of the percentages of *Campylobacter* spp detected in three commercial chicken flocks and viscera, a high level of presence of the bacteria found in lot 1, represented by 100% (16/16) detection, both as whole chickens viscera; in descending order lot 3, presented 75% of presence (12/16) in

the whole chickens and viscera 56.25% (9/16) and finally batch 2 was located with percentages *Campylobacter* spp 68, 75% (11/16) in chicken samples and 50% (8/16) for samples of viscera.

Table 3. Percentage Distribution of *Campylobacter* spp positivity in the viscera of chickens sold three lots in the municipality Girardot Aragua state, Venezuela.

% POSITIVITY A		
LOT	N° of Viscera	% in Viscera
1	16	100
2	16	50
3	16	56,25
TOTAL	48	68,75

A. The results are expressed as the percentage of positive rapid test viscera.

They were distributed individual and grouped different organs analyzed between the three commercial lots, noting that the viscera heart (18%) had the highest percentage of presence of *Campylobacter* spp, followed by liver-gizzard (14.58%), liver (12,5%), liver-heart (8.33%), gizzard (8.33%) and the lowest percentage stood the group consisting of viscera gizzard heart-values of 6.25%. (Table 4)

Table 4. Percentage distribution of the presence of *Campylobacter* spp giblets individual and grouped chickens, three lots traded in the municipality Girardot, Aragua, Venezuela.

VISCERA						
LOTS	Heart	Liver-gizzard	Liver	Liver heart-	gizzard	Heart-gizzard
1,2,3	9/48	7/48	6/48	4/48	4/48	3/48
	18%	14,583%	12,5%	8,333%	8,333%	6,25%

By measuring the degree of matching the extracted data from the study of the UFC variables in chicken and UFC viscera, we can see that the statistic Kendall as concordance coefficient for this study is under 0,163 and significance is (*) P<0.005, which indicated that between the two variables was statistically significant association, this statistical association reflects the correlation between the UFC and UFC variables in whole chickens in viscera (Table 5).

Table 5. Statistical correlation of the presence of *Campylobacter* spp in broilers and viscera three lots traded in the municipality Girardot, Aragua, Venezuela.

N	48
W de Kendall (a)	,163
Chi-cuadrado	7,811
Significance.	,005

Regarding the means of the colony forming units of *Campylobacter* spp detected in whole chickens and viscera, obtained in three batches of marketed products submitted to the detection of *Campylobacter* spp, the values were below the infective dose and found to lot 1 showed higher recovery values *Campylobacter* spp the infective dose (≥ 500 CFU) represented by 43.75% (7/16) for chickens and 25% (4/16) to the viscera, in lot 2 only 12.5% (2/16) for each of the samples of meat and offal presented above infective dose values, finally lot 3 with 6.25% (1/16) values greater than

500 UFC he found both in the flesh and viscera.

4. Discussion of Results

It may show that the rapid test detected the highest percentage of positivity of 81.25%, in whole chickens among the three batches of chicken carcasses studied (Table 2). These results can be attributed to the entry of birds to the processor from poultry production units with low management level, where feces of these birds, contaminated feathers, skin and different tanks used for processing plant as well as the post evisceration washing water leaving the microorganism trapped within the abdominal cavity and the skin as also noted [2] and [4]. These results are in more than 63% reports obtained from fresh chicken expended in Costa Rica range [3], [5], [11], to 71% reported in England by [2], [5], [14]. In Venezuela they were presented inferior results, 75% presence of *campylobacter* spp detected in whole chickens or their parts in Aragua state [8].

In relation to the viscera had a percentage lower than the results found in whole chickens in a 68.5% positivity, this may be due to more rigorous control in this area line profit in the different processing plants (Table 3). 68.5% These results are in lower proportions to 70.83% found by [8], liver and gizzard samples collected in expense of Aragua, Venezuela and equally low state when compared with those reported by [2], in liver of birds in Chile that reach 95.1% isolation of *Campylobacter* and Brazil that reach up to 78.7%.

These results reflect like other previous research that the *Campylobacter* spp is present in the birds that come to beneficiary plants and can often survive the different stages of processing, as specified [4] and should be taken programs into account in quality assurance

By measuring the degree of matching the extracted data from the study of the UFC, variables in chicken and giblets (Table 5). It is considered a new data, because in the literature reviewed no information related to the correlation of CFU of *Campylobacter* spp in broilers and viscera with which to conduct comparison found. The correlation evidenced in this study, is indicative of improper handling of carcasses and offal and reflects the degree of cross-contamination at different stages at the level of processing plants or benefit of birds as is argued by [2] and [5].

Regarding the means of the colony forming units of *Campylobacter* spp detected in whole chickens and viscera, obtained in three batches marketed analyzed; [8] reported 20% of samples chicken carcasses with over 500 U. F. C, inferior results to those found in this research for chicken samples values. This evaluation can deduce that chicken meat as a product of high consumption becomes for those who ingest it in a high-risk food, important in triggering the disease, since the largest number of UFC in relation to the viscera, if the handling and preparation of food at home is inadequate.

5. Conclusions

Detection of *Campylobacter* spp was 81.25% for chickens

and 68.50% for the viscera and found to lot 1 had the highest levels of contamination 100% for both samples, followed by batch 3 75% and 68.75% respectively for broilers and viscera, and the lowest values were found detection in batch 2 with 68.75% (chickens) and 50% (viscera), which is indicative of low hygienic conditions operability of chicken processing plants in the plots studied.

Statistical results indicate a correlation between samples of chickens and viscera of the lots analyzed, indicative of the association between colony forming units found in chickens with those found in the viscera and based on the infective dose of bacteria to some individuals (≥ 500 CFU) in order to cause disease risk was represented by 43.75% chickens and 25% of the viscera of lot 1, and 12.5% for the samples studied in the lot 2 and finally 6.25% of the samples lot 3.

It is observed that the hygienic conditions of the plants beneficiary chickens brands evaluated, lack of hygiene and control of critical points in the processing of products intended for human consumption.

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