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Quantitative Risk Assessment of Campylobacter in Whole Chicken During Retail in Changchun Region

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Abstract: Objective: In order to effectively prevent and control diseases caused by Campylobacter, we aim to understand the horizontal distribution and quantitative pollution of Campylobacter species in the retail process of broiler chickens in Changchun area, and provide data basis for quantitative risk assessment of Campylobacter pollution in the retail process of broiler chickens. Methods A total of 240 fresh and refrigerated whole chickens were collected from large supermarkets and farmers' markets around Changchun City. Using plate counting method for bacterial isolation and cultivation, and multiplex PCR method for typing and identification of Campylobacter. Results: A total of 26 positive samples were detected from 240 specimens, with the detection rate of 10.8%. The detection rate of samples, which contamination quantity of Campylobacter was more than 500 cfu/g, reached 2.1% (5 /240). The positive rate of samples in the farmers markets was higher than that in the supermarkets. Conclusion This study investigated the contamination level of Campylobacter during the retail process of whole chickens in Changchun area, providing data support for the risk assessment of Campylobacter contamination in chickens. Remind government regulatory authorities to strengthen the hygiene supervision of live poultry slaughterhouses in the agricultural product market, and improve the risk assessment of Campylobacter in the field of food safety.

Keywords: Campylobacter, Food Contamination, Risk Assessment, Quantitative Testing, Chicken

1. Introduction

Campylobacter is a zoonotic pathogen, which is a serious threat to human health. The prevention and control of campylobacter is of great public health significance [1]. Campylobacteriosis is one of the most important infectious diseases that is likely to challenge global health in the years to come. Campylobacter-associated enteric disease is estimated to be responsible for more than 160 million cases of gastroenteritis each year and is linked to growth stunting of infants living under conditions of poor sanitation and hygiene [2]. In some cases, C. jejuni may lead to the onset of Guillain–Barré syndrome, an autoimmune disorder characterized by acute and progressive neuromuscular paralysis [3].

The incidence and prevalence of campylobacteriosis have increased in both developed and developing countries over the last 10 years. The dramatic increase in North America, Europe, and Australia is alarming, and data from parts of Africa, Asia, and the Middle East indicate that campylobacteriosis is endemic in these areas, especially in children. In addition to C. jejuni, there is increasing recognition of the clinical importance of emerging Campylobacter species, including Campylobacter concisus and Campylobacter ureolyticus [4].

Poultry is a major reservoir and source of transmission of campylobacteriosis to humans. The comparative profiling analysis in this study successfully demonstrated that antibiotic-resistant and pathogenic strains of C. jejuni are highly prevalent on retail poultry [5]. In order to effectively prevent and control campylobacter disease, understand the pollution risk level of chicken campylobacter in the retail

process of broilers in Changchun area, and provide data support for food safety risk assessment. This study conducted a survey on campylobacter contamination in broilers sold in large supermarkets and farmers' markets around Changchun City from September 2018 to August 2019.

2. Materials and Methods

2.1. Sample Collection

From September 2018 to August 2019, 20 retail frozen or freshly slaughtered whole chickens were collected every month from major supermarkets and farmers' markets around Changchun City. Collect whole chicken samples on the day of the experiment and place them in Stomacher 3500 ® Record the detailed information of the sample (collection location, weight, type, etc.) in the homogenization bag after sealing, and transport it back to the laboratory after refrigeration. Complete the testing within 2 hours after sample collection.

2.2. Instruments and Reagents

Electronic balance (sensitivity: 0.01 g), heating block, Thermostatic incubator, PCR instrument, QIAxcel capillary electrophoresis instrument, microaerobic production bag (3.5 L, produced by OXOID company in the UK), sterile homogenization bag (stomacher 3500 ®, Seward Company, UK).

Buffered peptone water (BPW), Wang's culture dish, Sterile lysis and defibration of sheep blood, Cefoperazone additive, polymyxin B additive, rifampicin additive (Beijing Land Bridge Technology Co., Ltd) Muller-Hinton culture dish, Preston culture dish, Karmali culture dish, Selective additiveand selective additive SR0205E, SR0204E (Oxford Company in the UK); Campylobacter multiplex PCR reagent (Huizhi Taikang Biotechnology Co., Ltd, Batch number: 201807); QIAxel DNA Screening Kit2400, 50-800bp Size Marker, 15-1000bpAlignment Marker (QIAGEN, in the UK.).

2.3. Quantitative Detection and Identification of Campylobacter in Samples

2.3.1. Sample Process

Place the chicken carcass sample in a sterile homogenization bag, add 500 mL/kg sterile buffer peptone water under sterile operating conditions, and repeatedly rub the chicken carcass sample for 5 minutes; Homogeneous. Take 1 mL of sample eluent and place it in a 9 mL sterile saline test tube to prepare a 1:10 dilution of the sample; Take 1 mL of 1:10 times sample dilution and prepare a 1:100 times sample dilution. Take 100 samples of the original solution of the rinsing solution, 1:10 times, and 1:100 times of the sample diluent, respectively μ L. Immediately apply to Preston and Karmali platesculture dishes, with 2 parallel dilutions, and incubate at 42°C for 48 ± 2 hours in a microaerobic environment (5% CO_2, 10% CO_2, 85% N_2).

2.3.2. Colony Count

When no Proteobacterium colonies appear on the Karmali plate, the plate with less interference from foreign bacteria can be selected from two different media for Campylobacter counting and reporting. When Proteus colonies appear on the Karmali plate, count and report Campylobacter based on the culture results on the Preston plate. After 2-3 days of cultivation, select a dilution with an average suspected colony count of 15-150 CFU/dish for counting. If the average number of suspected colonies on each dilution plate is not within the range of 15-150 CFU/plate, and if the average number of suspected colonies on all dilution plates is less than 15 CFU/plate, select the suspected colonies on the lowest dilution plate for counting; If the number of suspicious colonies on all dilution plates is>150cfu/plate, select the suspicious colony on the highest dilution plate for counting. If no suspected colony growth is found in all dilutions, continuous cultivation for 7 days is required before discarding the initial separation plate.

2.3.3. Bacterial Isolation and Identification

Preliminary identification of bacteria in combination with GB 4789.9-2014 [7], using an inoculation needle, select at least 5 suspected colonies from two plates of the same dilution. When the total number of colonies is less than 5, all of them are selected and inoculated onto MH blood plates with lines. Incubate at 42°C for 48 hours ± 2 hours under microaerobic conditions. Extract genomic DNA from suspicious pure bacterial colonies and use Multiplex-PCR method for identification. Select QIAxcel capillary electrophoresis instrument Method Details AM420 for amplification product analysis. All Campylobacter genera can detect 650 bp amplified fragments (internal reference: 23SrRNA), 323 bp amplified fragments of Campylobacter jejuni, and 126 bp amplified fragments of Campylobacter coli.

2.3.4. Result Representation of Colony Count

Based on the PCR identification results, calculate the actual number of campylobacter colonies selected in the sample, multiply by the dilution factor, and report the number of campylobacter colonies per g of sample, expressed in CFU/g; If there are no suspicious colonies or confirmed campylobacter in all dilutions, report at a dilution ratio of less than 1 times the minimum dilution factor.

The calculation formula is as follows: the actual number of campylobacter colonies on each plate=the average number of suspicious colonies on two plates × (PCR result positive colony count/suspicious colony count picked).

2.3.5. Preservation and Transportation of Bacterial Strains

Select single colonies of Campylobacter that have been identified as positive by PCR on MH blood plates, line them and inoculate them on MH blood plates. After 48 hours of cultivation in a microaerobic environment at 42°C, use sterile cotton swabs to calculate the formula as follows: the actual number of Campylobacter colonies on each plate=the average number of suspicious colonies on both plates × (PCR result positive colony count/suspicious colony count picked).

After wiping all the bacterial moss on the MH blood plate, store it in a frozen tube containing Wang's culture medium for

bacterial species preserva.

3. Results

3.1. Qualitative Detection Results of Campylobacter in Whole Chicken Samples

A total of 240 commercially available whole chickens were tested throughout the year, and 26 positive samples of Campylobacter were detected, with a positive detection rate of 10.8% (26/240). Throughout the year, Campylobacter was only detected in September and December 2018, and in May and August 2019, with a positive rate of 10% to 55%. However, it was not detected in other months. Among them, 25 samples detected Campylobacter jejuni. Campylobacter coli was detected in 6 samples; It is worth noting that both Campylobacter jejuni and Campylobacter colon were detected in 5 samples.

3.2. Quantitative Detection Results of Campylobacter in Whole Chicken Samples

Quantitative testing was conducted on 240 whole chicken samples sold in the market, and campylobacter was detected in 26 samples. The detection rate of campylobacter in samples from agricultural markets was much higher than that in samples from large supermarkets (χ^2 =22.21, P<0.01). The contamination level of campylobacter in 26 positive samples ranges from 3~1700 CFU/g, the contamination level of campylobacter in samples from agricultural markets ranges from 3~1700 CFU/g, and the contamination level of campylobacter in samples from supermarkets ranges from 13~700 CFU/g. Samples with campylobacter contamination levels>500 CFU/g accounted for 2.1% (5/240), all from agricultural markets. The contamination level of campylobacter in samples from agricultural markets is higher than that of samples from large supermarkets. See Table 1.

Table 1. *Contamination of Campylobacter in retail chicken in Changhun.*

sample source	N	positive sample		Colony count (CFU/g)			χ^2	*P*
		n	r/%	median	Min	Max		
a market of farm produce	92	21	22.8	51	3	1700	22.21	<0.01
Supermarket	184	5	3.4	23	11	700		
total	240	26	10.8	50.5	3	1700		

4. Conclusion

This study collected 240 whole chicken samples for sale in Changchun area from September 2018 to August 2019, and quantitatively evaluated the carrier status of Campylobacter in whole chicken samples during the retail process. The positive rate of Campylobacter in the whole chicken sample during the retail process was 10.8% (26/240), with the positive rates of Campylobacter jejuni and Campylobacter colon being 10.4% (25/240) and 2.5%, respectively (6/240) (6/240). The detection rate of Campylobacter in whole chicken samples from farmers' markets was 22.8% (21/240), while the detection rate in samples from supermarkets was 3.4% (5/240), with statistical significance (χ^2=22.21, P<0.01). In terms of pollution level, the total pollution level of Campylobacter in 26 positive samples is 3~1700 CFU/g, among which the pollution level of samples from agricultural markets is 3~1700 CFU/g,, and the pollution level range of samples from large supermarkets is 13~700 CFU/g. 2.1% (5/240) of the samples with campylobacter contamination levels>500 CFU/g in the whole chicken were from agricultural markets. From the source of the samples, the contamination rate and level of campylobacter in the samples collected from agricultural markets are higher than those in large supermarkets.

5. Discussion

In recent years, food safety problems caused by Campylobacter have become increasingly prominent, and the incidence rate of Campylobacter has increased in the world. Therefore, more and more countries are paying attention to monitoring and risk assessment of Campylobacter contamination in edible poultry. The World Health Organization assesses the global and regional disease burden of 22 foodborne bacteria, protozoa, and viral diseases. In order to estimate the disease burden caused by contaminated food, according to global experts' inference, the foodborne diseases caused by Campylobacter genus are 96 million (95% UI 5.2~177 million) [7].

It is generally believed that consuming contaminated chicken products is the most common cause of Campylobacter infection [8]. Research has shown that handling and consuming raw or undercooked poultry meat is the main cause of human infection with Campylobacter [9]. The colonization of campylobacter in the chicken intestine can reach up to 10^6~10^9CFU/g and remain in this infected state until slaughter [10]. The average prevalence of campylobacter in European chicken flocks is as high as 70%, while it varies from 2% to 100% worldwide [11].

The results of this study show that the carrier rate of Campylobacter in the retail process of whole chickens in Jilin Province is lower than that in other regions, which may be due to the fact that survey sampling is mostly conducted in autumn and winter or at a distance. Lower temperatures cause damage to the growth of Campylobacter jejuni, resulting in a low detection rate. In addition, due to the impact of avian influenza, there were no fresh whole chickens sold in the surrounding markets of Changchun from January to April 2019, and frozen chicken may have been purchased, which has a significant impact on the detection rate.

As a foodborne pathogen, C. jejuni encounters various stress conditions at different stages of food production, processing, preservation, distribution, and cooking, and

aerobic stress would be a common stressor to this microaerophilic bacterium in the normal atmosphere [5]. Campylobacter jejuni is more sensitive to the external environment and no longer grows below 30°C [12], entering a live non cultivable state (VBNC) [13]. Research has confirmed that the number of Campylobacter jejuni on the surface of chicken meat decreases during refrigeration [14, 15, 16], and the number of Campylobacter jejuni on the surface of frozen chicken meat also decreases [17].

In addition, Studies have shown that drug-resistant and epidemic strains of Campylobacter jejuni are more effective in surviving on refrigerated raw chicken in the air than sensitive strains [18], the high prevalence of drug-resistant and epidemic strains of Campylobacter jejuni in retail poultry may affect food safety [19].

There is also some uncertainty in this study: the purpose is to conduct a preliminary exploration of quantitative microbial risk assessment in the retail process of broiler chickens. Based on the data collected from retail broiler chickens in Changchun area, the feasibility of risk assessment of Campylobacter in the retail process of broiler chickens is determined, providing reference for future correlation research. It is suggested that government regulatory authorities should strengthen the hygiene supervision of live poultry slaughter points in agricultural markets, improve the risk assessment of campylobacter, and minimize the health hazards it causes. At the same time, in order to promote the development of quantitative risk assessment for important foodborne pathogenic bacteria such as Campylobacter in China, it is necessary to supplement and improve the corresponding basic data of each link to improve the accuracy and application value of quantitative risk assessment.

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Biography

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