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Prevalence and quantification of *Salmonella* contamination in raw chicken carcasses at the retail in China

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ABSTRACT

The quantitative contamination load of *Salmonella* in raw chicken carcasses at the retail level in six provinces and cities of China was determined within 1595 carcasses over 12 consecutive months. The overall *Salmonella* contamination rate was 41.6% and the median load of those contaminated was 4.6 MPN/100 g with 1.8 MPN/100 g as the 25th percentile and 18.0 MPN/100 g as the 75th percentile. There were significant variations in prevalence among carcasses sampled either in different provinces or sampling months. Carcasses collected in August had not only the highest prevalence of contamination (55.8%), but also the highest median (14.0 MPN/100 g) and 75th percentile load (120.0 MPN/100 g) values compared to January with lowest prevalence (26.5%), median (1.5 MPN/100 g) and 75th percentile load (7.6 MPN/100 g). The chilled (55.1%) stored carcasses was significantly higher in prevalence than those frozen (33.5%) and those freshly slaughtered (28.3%), those unpackaged (45.1%) was more likely to be contaminated with *Salmonella* than those packaged (37.4%). The present study revealed the widely prevalent *Salmonella* contamination among retail carcasses, indicating a strong potential of the cross-contamination occurred before and/or at the retail level. The study also provided quantitative data for a risk assessment evaluating potential intervention methods to reduce the risk of salmonellosis by consuming chicken meat of Chinese origin.

1. Introduction

Salmonellosis is one of the major foodborne diseases in the world and it is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* species occur globally each year, with 155,000 deaths (Majowicz et al., 2010). There is a wide range of foods implicated in foodborne illness attributable to *Salmonella*. Foods of animal origin, especially poultry and poultry products, are often involved in sporadic cases and outbreaks of human salmonellosis (Sánchez-Vargas, Abu-El-Haija, & Gómez-Duarte, 2011).

In last decades, there had been several quantitative microbiological risk assessments (QMRA) studies for *Salmonella*-chicken meat combinations including the whole or part of the poultry food chain performed in the world, which showed the strong linkages between the prevalence and density of *Salmonella* infection in chicken meat and human cases of salmonellosis (Guo et al., 2011; Oscar, 2004; Straver et al., 2007; World Health Organization and Food and Agriculture Organization of the United Nations, 2002). Control of *Salmonella* in food animals had been successful in the United States (M'ikanatha et al., 2010), Sweden and Denmark (Wegener et al., 2003) and had led to low levels of salmonellosis in these countries.

China has a high case rate of salmonellosis. It was found that non-typhoid *Salmonella* annually caused 9.874 million gastroenteritis cases in China and 91.5% of them were caused by food

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transmission (Mao, Hu, & Liu, 2011). It had been found that more than half of the retail chicken carcasses in China were contaminated with *Salmonella* (Yang et al., 2011), however, the quantitative load among those contaminated chicken meat was still unknown which made both scientists and government unable to evaluate the risk of salmonellosis among Chinese populations and explore the potential interventions under Chinese dietary habits. The aim of the study was to determine the prevalence and loads of *Salmonella* on retail raw chicken carcasses in parts of China and to provide the data for quantitative risk assessments of *Salmonella*.

2. Materials and methods

2.1. Sample collection and preparation

From April 2011 to March 2012, 1595 samples including freshly slaughtered, chilled and frozen chicken carcasses were collected from supermarkets and farmer's markets in Beijing, Changchun of Jilin province, Huhehaote of Inner Mongolia Autonomous, Yanglin of Shanxi province, Yangzhou of Jiangsu province and Guangzhou of Guangdong province, respectively. Each sample was weighed, marked, placed in a 3500 mL stomach (Seward, UK) plastic bag and was transported to local laboratories immediately in an ice box. 500 mL of buffered peptone water (BPW; BD, Beijing, China) per kilogram of chicken sample was added and the carcass was thoroughly manually massaged for 3–5 min to ensure the surface, internal and external of the chicken carcass fully contact with the rinse. The rinse was used for *Salmonella* MPN analysis. The whole analysis process should be finished in no more than 2 h after sampling in order to avoid the potential growth of *Salmonella* during sample storage and transportation.

2.2. Salmonella MPN

The analytical procedure for *Salmonella* mainly followed the Food Safety and Inspection Service (FSIS) of United States Department of Agriculture (USDA/FSIS, 2008, 2010) with a bit modification. Briefly, a triplicate-10 mL of the chicken rinse was taken directly from the stomach bags and placed into three empty sterile tubes, transferring triplicate-1 mL of the rinse to three tubes containing 9 mL of BPW followed by making 10-fold dilution, respectively. All tubes were pre-enriched in a shaking incubator at 100 rpm/min for 20–24 h at 37 °C. A portion (0.5 ± 0.05 mL) or 0.1 ± 0.02 mL of the pre-enrichment culture was transferred into 10 mL tetrathionate broth (TT; BD) or Rappaport Vassiliadis broth (RV; BD) and incubated at 42 ± 0.5 °C in a shaking incubator at 100 rpm for 22–24 h for selective enrichment. After incubation, a loopful of TT and RV broth culture was streaked onto xylose lysine tergitol 4 agar (XLT4; BD) and incubated at 37 ± 1 °C for 22–24 h. Colonies of presumptive *Salmonella* on XLT4 plate were selected and purified on XLT4 again and incubated at 37 ± 1 °C for 20–24 h. No more than one colony with typical *Salmonella* phenotypes was inoculated onto Luria–Bertani agar and confirmed by API 20E test kit (bioMérieux, Beijing, China). The MPN of each sample would be acquired by searching the table of MPN index and 95% confidence limits for various combinations of positive tubes in a three dilution series using inoculums quantities of 10 mL, 1 mL and 0.1 mL recommended by the FSIS of United States Department of Agriculture. Since each kilogram of chicken sample was rinsed in 500 mL BPW, MPN/100 g of chicken carcass was equal to the MPN of 50-mL chicken rinses (Wang, Yu, & Liu, 2005).

2.3. Statistical analysis

The Pearson Chi-Square test and the nonparametric test (Mann–Whitney Test for two independent samples and Kruskal Wallis Test for several independent samples) were used to determine the significant difference of prevalence and median loads of *Salmonella* in chicken carcasses sampled in different provinces, months, storage conditions, packaged conditions, and market types. Statistical software SPSS for Windows (version 11.5, SPSS, Inc., Chicago, IL) was used for descriptive analysis. The results were considered significant at the 5% (with $\alpha = 0.05$) level to evaluate whether there is any association between the prevalence and quantifications of *Salmonella* contamination and their characteristics at the retail.

3. Results

3.1. Overall Salmonella prevalence and MPN determination

There were totally 1595 chicken carcasses and 41.6% of them found to be positive of *Salmonella* contamination. The quantitative microbiological method adopted in the present study used most probable number (MPN) as the unit to quantify load values of the contaminated chicken carcasses and the range was from 1.5 MPN/100 g to 550 MPN/100 g. The median value of *Salmonella* loads among those positive samples was 4.6 MPN/100 g. The 25th percentile (Q1) and the 75th percentile (Q3) of the contamination were 1.8 MPN/100 g and 18.0 MPN/100 g, respectively.

3.2. Varied prevalence and quantification of Salmonella contamination among chicken carcasses from different provinces and months

The prevalence of *Salmonella* among chicken carcasses sampled from different provinces varied significantly (Table 1) with the highest contamination rate from Jilin province (65.0%) and the lowest from Inner Mongolia Autonomous (15%). However, the quantification of *Salmonella* in terms of median MPN values among those positive carcasses was found highest from Guangdong provinces, 7.5 MPN/100 g (1.8 MPN/100 g for Q1 and 21.5 MPN/100 g for Q3), which was more than 4 times higher than lowest median values found in carcasses from Inner Mongolia 1.8 MPN/100 g (1.8 MPN/100 g for Q1 and 4.6 MPN/100 g for Q3). The Kruskal Wallis Test observed significant difference in quantifications of *Salmonella* contaminations among those positive carcasses collected from different provinces ($P < 0.001$).

The present study also observed significant differences both in prevalence and quantification among those carcasses collected in different seasons ($P < 0.001$) in Table 2. The prevalence among those carcasses collected in summer, including those in June, July and August, was significantly higher than those collected in autumn (including September, October and November, $P = 0.001$) and those collected in winter (including December, January and February, $P < 0.001$), but not significantly different from those collected in spring (including March, April and May, $P = 0.177$). Using Mann–Whitney Test, the median load value (MPN/100 g) of carcasses collected in summer, 8.0 MPN/100 g, was all significantly higher than those collected in spring (4.6 MPN/100 g, $P < 0.001$), autumn (4.6 MPN/100 g, $P = 0.005$) and winter (3.6 MPN/100 g, $P < 0.001$), respectively.

Fig. 1 showed varied prevalence of *Salmonella* contamination among carcasses collected in different months with significant difference ($P < 0.001$) using the Pearson Chi-Square Test. The prevalence increased from January to August and decreased from then to December and those collected in August had higher