Listeria monocytogenes in retailed raw chicken meat in Malaysia


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ABSTRACT This study aimed to determine the prevalence Listeria monocytogenes in raw chicken meat samples at hypermarkets and wet markets. Chicken drumsticks, breasts, and thighs were randomly selected. The most probable number (MPN) PCR method was used to quantify the L. monocytogenes in the samples. Listeria monocytogenes was detected in 20% of the samples. Occurrence of L. monocytogenes was highest in breast (42.03%) followed by drumstick (11.27%) and thigh (7.14%). Samples from hypermarkets showed higher occurrence (25.71%) of L. monocytogenes compared with wet markets (14.29%). The density of L. monocytogenes found in samples ranged from <3.0 to 16 MPN·g⁻¹. The presence of L. monocytogenes in raw chicken meat is unwanted but unpreventable. Thus, further research on the processing method to reduce and eliminate this kind of bacteria in chicken meat before consumption is necessary. The presence of L. monocytogenes in chicken samples suggests the importance of this pathogen in chicken. Thus, more study is needed to find ways to eliminate this pathogen from poultry.

Key words: Listeria monocytogenes, foodborne, prevalence, raw chicken meat, most probable number PCR

INTRODUCTION

Listeria monocytogenes is a gram-positive, non-spore-forming, and facultative anaerobic bacterium. Listeria monocytogenes can be found ubiquitous in the environment including water, soil, human, and animal feces as well as variety of food. Food-borne listeriosis is a relatively rare infection caused by L. monocytogenes. The occurrence rate of listeriosis is about 5 cases per million people per year in countries where listeriosis is statutorily notifiable, for instance, France, Germany, and Switzerland (Garrido et al., 2008). Listeriosis primarily strikes immunocompromised individuals with a fatality rate of 20 to 25% (Hitchins and Whiting, 2001). However, listeriosis has high mortality rate compared with other food-borne disease (Osaili et al., 2011). From a food safety point of view, L. monocytogenes is an important pathogen due to its ability to grow at refrigeration temperatures and tolerate osmotic stress (Rhoades et al., 2009).

A listeriosis outbreak occurred in United States in 2011, linked with consumption of contaminated cantaloupe. A total of 146 persons infected and 30 deaths have been reported (CDC, 2011). In 2008, there were listeriosis cases confirmed in Canada and 22 deaths reported (CBC News, 2009). Poultry can be contaminated with L. monocytogenes during production, in the processing plant, and in storage, and chicken products can become contaminated through contaminated raw material or cross-contamination during preparation, cooking, and serving food (Mylius et al., 2007; Osaili et al., 2011).

Chicken meat is a popular and important source of protein in Malaysia. Poultry meat consumption has increased since 2001 (Department of Veterinary Services Malaysia, 2011). Contamination of poultry with L. monocytogenes is unpleasant. Listeria monocytogenes is able to survive during processing techniques and increase chances of cross-contamination for other foods (Barbalho et al., 2005; Goncalves et al., 2005).

Conventional methods for detection of L. monocytogenes involve selective enrichment, followed by subsequent biochemical tests, which are laborious and time-consuming (Almeida and Almeida, 2000). It is also difficult to determine the number of L. monocytogenes due to high levels of competitive microorganisms (Capita et al., 2001). Thus, the most probable number (MPN) method is usually used for quantification of L. monocytogenes because the MPN method is suitable for quantification of low level of microflora (≤10 to 100
MPN-g⁻¹; Martín et al., 2004). Polymerase chain reaction is applied to increase sensitivity and specificity of MPN method by detecting the presence of L. monocytogenes virulence factors such as phosphotyrosinolipid phospholipase C (ple A), hemolysin (hly A), invasive associated protein (iap), and actin polymerization protein (act A; Furrer et al., 1991; Portnoy et al., 1992).

Recently, MPN-PCR method always applied for rapid and effective detection and quantification of foodborne pathogen (Chai et al., 2007; Jeyaletchumi et al., 2010; Pui et al., 2011). The objective of this study was to determine the prevalence of L. monocytogenes in raw chicken meat samples available at hypermarkets and wet markets in Malaysia using the MPN-PCR method.

MATERIALS AND METHODS

Sample Collection

A total of 210 samples of raw chicken meat (71 drumsticks, 69 breasts as well as 70 thighs) were collected randomly from 3 hypermarkets (A, B, and C) and 3 wet markets (D, E, and F) in Selangor area, Malaysia, over a period of 5 mo (September 2011 to January 2012). A hypermarket is a very large retail store selling full lines of general merchandise and groceries, whereas a wet market is an open fresh food market. Sampling was done in Selangor, Malaysia, which consists of an appreciable urban and rural population. All the wet markets and hypermarkets in the state were listed, and 3 hypermarkets (A, B, and C) and 3 wet markets (D, E, and F) were selected with a random number table for this study (Jeyaletchumi et al., 2010). Samples were transported to the laboratory, and analysis was carried out immediately.

Detection and Enumeration of Listeria Monocytogenes by MPN-PCR

Examination of chicken meat was carried out according to FDA-BAM Standard for Listeria detection (Hitchins and Jinneman, 2011) with the minor modification proposed by Wong et al. (2011) and Jeyaletchumi et al. (2010) at the enrichment stage [Listeria enrichment broth (LEB; Merck, Darmstadt, Germany) was used in the enrichment stage instead of buffered LEB], as well as inclusion of PCR for detection.

Briefly, a 10-g sample was mixed with 90 mL of LEB for 90 s. The mixture was incubated for 4 h at 30°C. After the preenrichment step, the selective agents acriflavine with final concentration of 10 mg·L⁻¹, cycloheximide with final concentration of 50 mg·L⁻¹, and nalidixic acid with a final concentration of 40 mg·L⁻¹ were added. The 10-fold (10⁻¹) enriched broth was made up to 100-fold (10⁻²) and 1,000-fold (10⁻³) dilutions using LEB. For the 3-tube MPN method, each dilution (1 mL) was transferred into triplicate MPN tubes (Sutton, 2010). Tubes were incubated at 30°C for 48 h. All measurements were carried out in triplicate.

DNA Extraction

The MPN tubes were examined for turbidity and turbid tubes were subjected to DNA extraction using the boiled cell method (Jeyaletchumi et al., 2010). Briefly, centrifugation at 10,000 × g (5 min at ambient temperature) was carried out to pellet the microorganisms. The supernatant was discarded, and the pellet was resuspended in 500 µL of sterile distilled water and boiled for 10 min. The tubes were then subjected to cooling at −20°C (10 min) followed by centrifugation at 10,000 × g (10 min at ambient temperature). Supernatants were used as DNA template for detection of L. monocytogenes.

Primers and PCR Conditions

For detection of L. monocytogenes, 2 primer pairs were used to identify 16S rRNA gene (938 bp region) and hlyA gene (702 bp). The sequences of the primer pair used for targeting 16S rRNA were 5'-CTC CAT AAA GGT GAT CCT 3' and 5'CAG CCG CCG TAA TWC-3', whereas primers pair used for targeting the hlyA gene were 5'-CCT AAG ACG CCA ATC GAA-3' and 5'-AAG CGC TTG CAA CTG CTC-3' (Jeyaletchumi et al., 2010). The PCR amplification was performed in 25 µL of a reaction mixture that consisted of 5× PCR buffer, dNTP (10.0 mM), MgCl₂ (25 mM), 500 U Taq DNA Polymerase, 10 pM of each primer, sterile distilled water, and 2 µL of DNA template. Amplification was performed on a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA) with the following conditions: initial denaturation at 94°C for 5 min for 1 cycle, 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 53°C for 1 min and elongation at 72°C for 2 min, and a final extension at 72°C for 7 min. In all the reactions, the negative control (without DNA) and standard L. monocytogenes (ATCC 19115) as positive control were included to ensure the amplifications are true positive.

Next, 5 µL of the PCR products was electrophoresed on a 1.0% agarose gel at 100 V for 28 min. A DNA molecular ladder (Vivantis Technologies, Subang Jaya, Malaysia) was included in each gel. The agarose gel was stained with ethidium bromide and visualized under UV light.

Data Analysis

All measurements were carried out in triplicate. Minitab (v. 14) statistical package (Minitab Inc., State College, PA) was used to determine if there was any significant difference between prevalence of L. monocytogenes in chicken meat among the sampling locations (hypermarkets and wet markets; P < 0.05).

RESULTS

Results showed that 42 out of 210 chicken samples were contaminated with L. monocytogenes (20%). As
shown in Table 1, the occurrence of *L. monocytogenes* was highest in breast (42.03%) followed by drumstick (11.27%) and thigh (7.14%; *P* < 0.05).

Samples from hypermarkets showed significantly higher occurrence of *L. monocytogenes* in chicken meat, 25.71%, compared with wet markets, 14.29% (Table 1; *P* < 0.05). However, Arumugaswamy et al. (1994) reported that the prevalence of *Listeria* between the supermarket and wet market in chicken samples did not differ significantly and 60% of raw chicken portions were contaminated with *L. monocytogenes*.

Microbial load (MPN·g⁻¹) of *L. monocytogenes* in the samples was enumerated using MPN-PCR method. Results from MPN-PCR showed that the microbial load of *L. monocytogenes* in chicken samples ranged from <3 to 16 MPN·g⁻¹ (Table 2). The microbial load found in this study was not very high.

**DISCUSSION**

The occurrence of *L. monocytogenes* in this study was found to be similar to the results reported by van Nierop et al. (2005). van Nierop et al. (2005) found 19.2% of the chicken carcass samples from retail outlets in South Africa were contaminated with *L. monocytogenes*. Incidence of *L. monocytogenes* in fresh chicken from street vendors and supermarkets was 16.7 and 20%, respectively (van Nierop et al., 2005). Lewis and Corry (1991) reported that 56% of the raw chicken samples in the United Kingdom are contaminated with *L. monocytogenes*. Osaili et al. (2011) reported that the prevalence of *L. monocytogenes* in raw broiler chicken was 9%. Other studies showed high percentages of *L. monocytogenes* (36.1, 60, 11.5, and 21.6%) from raw chicken in Spain, Portugal, Turkey, and Italy, respectively (Mena et al., 2004; Yucel et al., 2005; Vitas et al., 2007; Pesavento et al., 2010).

As mentioned by Osaili et al. (2011), the food safety regulations of some countries (e.g., United States, Australia, New Zealand) require zero tolerance of *L. monocytogenes* in ready-to-eat food. The prevalence of this pathogen in poultry and meat products generally ranges from 2.7 to 20%, whereas the prevalence of *Listeria* spp. ranges from 1.8 to 48% (Osaili et al., 2011).

In the United States, zero tolerance (in 25 g of food) is required, whereas the European Commission permits a limit of 10 to 100 cfu·g⁻¹ (European Commission, 1999). However, there is no zero limit of enumeration for *L. monocytogenes* in Malaysia.

There have been some studies on the occurrence of other pathogens (e.g., *Salmonella*) in a variety of foodstuffs consumed in Malaysia. Based on the latest report by Pui et al. (2011), the maximum number of 19 MPN·g⁻¹ for *Salmonella* in sliced fruits is considered high because such products are consumed in raw form. Because chicken meat is processed or cooked before consumption, the presence of *L. monocytogenes* is not as serious as fruits or ready-to-eat foods.
The variation in prevalence of *L. monocytogenes* in samples from hypermarkets and wet markets might be due to the differences in holding time and processing ways of the food before sale. In hypermarkets, chicken meat is usually stored in cold rooms before being displayed on ice, whereas in wet markets, chickens were slaughtered in the morning and stored on ice cubes before being sold. Thus, the holding time of chicken in hypermarkets was relatively longer than wet markets.

The relatively longer holding time before retailing is a major factor for high prevalence of *L. monocytogenes* because this microorganism is able to survive in low temperatures (Walker et al., 1990) and tolerate cold stress (Schmid et al., 2009). An initial contamination as low as 1 cfu of *L. monocytogenes* per 100 g can make the food unsafe within 32 d, whereas 10 cfu-g⁻¹ can make the food dangerous within 8 d (Salvat and Fravalo, 2004). So, it is not surprising that *L. monocytogenes* in samples from wet markets was not as prevalent as that of hypermarkets.

In addition, the difference in prevalence of *L. monocytogenes* in different parts of chicken might be due to the variation in water content and nutrient level of chicken parts. Montville and Matthews (2008) stated that listerial growth is affected by humidity and nutrient contents of the food.

The presence of *L. monocytogenes* in chicken samples suggests the importance of this pathogen in chicken. Thus, more study is needed to find ways to eliminate this pathogen from poultry.

**CONCLUSIONS**

Presence of *L. monocytogenes* in raw chicken meat is undesirable but unavoidable. Thus, further research on the processing method to reduce and eliminate this kind of bacteria in chicken meat before consumption is necessary.

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