Risk of acquiring listeriosis from consumption of chicken offal among high risk group

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Abstract

Listeria monocytogenes causes mild foodborne infection in healthy adults but serious complication in high risk group such as infant, pregnant women, immunocompromised and elderly. This study aims to estimate the probability of infection based on the survivability of L. monocytogenes in chicken offal, particularly chicken liver, gizzard and heart. A total of 20 µl of L. monocytogenes with inoculum size of 9.02 log CFU/ml was spiked on the chicken offal and stored at room temperature (28°C) for 0, 1, 3, and 6 h. This experimental design was aimed to simulate the contaminated chicken offal stored at temperature danger zone. It was found that chicken offal supported the growth of L. monocytogenes that it significantly grows at 3 and 6 h incubation. Risk assessment was performed by combining this result with the data from our previous studies as well as data from government agencies and other studies. Dose-response model was used to estimate the probability of listeria infection per year. Immunocompromised was the highest risk group, followed by pregnant women, elderly and general population with probability of infection per year estimated at 3.78 x 10⁻³, 1.76 x 10⁻⁴, 5.68 x 10⁻⁸ and 5.75 x 10⁻⁸, respectively. It can be concluded that high risk group requires extra caution in their food consumption in order to prevent listeriosis which can result in serious complication and death.

Keywords: Risk assessment, L. monocytogenes, chicken offal, immunocompromised, pregnant women, elderly

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INTRODUCTION

The genus Listeria includes six different species, L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri and L. grayi. Both L. monocytogenes and L. ivanovii are pathogenic, but only L. monocytogenes is associated with humans and animal illness (Rodríguez-Lázaro et al., 2004). L. monocytogenes has emerged as a foodborne pathogen and implicated with various food ranging from dairy products, fresh produce, meats and seafoods. High risk groups such as pregnant woman, immunocompromised individuals, young children and elderly are normally suffer from serious listeriosis infection through consumption of contaminated foods (EFSA, 2018; Norton and Braden, 2007). Cases of L. monocytogenes are rare as shown in the United States with estimated 0.26 cases per 100,000 individuals for the year of 2013 (Nastasijevic et al., 2017). However, the severity of listeriosis accounted for about 30% more than other common pathogens which resulted in high hospitalization rates and mortality (Crim et al., 2014). Listeria has also been implicated with outbreaks caused by contaminated ice cream (CDC, 2015a) and cheese (CDC, 2015b) and it caused the highest number of death in Europe in the period of 2008-2012 (EFSA and ECDC, 2014). Recently, L. monocytogenes has also implicated with outbreak related to pork products which cause four people hospitalized (CDC, 2018).

Foodborne diseases cause significant social and economic burdens from productivity losses, cost of medical treatment and hospitalization (Jeyaletchumi et al., 2010). Risk analysis is an important model to improve food safety by determining the estimated risk of infection in a particular community exposed to the pathogen (Marvin et al., 2009; Jeyaletchumi et al, 2010). Foodborne illness can occur from poor hygiene practices by food handlers as well as consumer. Those who do not practice proper food handling are potentially transmitted pathogens to food due to cross contamination from food contact surfaces (Soon, 2019). Study shown food handlers were not practicing the food safety knowledge they have acquired (Zanin et al., 2017).

Chicken has been the major source of protein in Malaysia compared to beef, pork, duck and lamb. The per capita consumption of chicken has been steadily increasing from 38.59 kg in the year 2007 to 50.32 kg in year 2016 (DVS, 2018). Besides chicken meat, chicken offal also serves an important part of Malaysian food. Our previous studies have shown L. monocytogenes present in both chicken meat and chicken offal at an average of 20% and 24 %, respectively (Goh et al., 2012; Kuan et al., 2013). The results show a significant percentage of chicken meat and chicken offal were contaminated and posed risk of listeriosis among high risk group.
in a susceptible individual. In the present study, a risk assessment model was developed to determine the risk of acquiring listeriosis based on the survivability of \textit{L. monocytogenes} on chicken offal.

**METHODOLOGY**

**Statement of purpose**

The goal of this study was to estimate the probability of listeria infection from chicken offal consumption based on the \textit{L. monocytogenes} survivability on chicken offal. The calculation of risk estimate was based on data from this study, previous study (Goh et al., 2014), and assumptions based on the data from other studies and government data. All the assumptions and uncertainties surrounding inputs were clearly stated. Risk assessment model was developed based on hazard identification, hazard characterization, exposure assessment and risk characterization. The probability of listeriosis from chicken offal consumption was calculated.

**Hazard identification**

\textit{L. monocytogenes} can be found in a diverse environment, food products, animals and human (Buchanan et al., 2017). Almost all cases of human listeriosis are foodborne which are contaminated with \textit{L. monocytogenes} (Cossart and Bierne, 2001). Studies have found \textit{L. monocytogenes} present in chicken offal, such liver and gizzard averaging from 26 to 63 (Arumugaswamy et al., 1994; Kuan et al., 2013). Previous risk assessment study concluded that most listeriosis cases happened to the susceptible groups after the consumption of food that supports growth stored at inappropriate temperature for an extended period of time (US FDA/FSIS, 2003). Based on the prevalence study, chicken offal consumption has been reported to pose risk of listeriosis among high risk group (Kuan et al., 2015).

**Hazard characterization**

\textit{L. monocytogenes} is the bacteria species that are predominantly associated with human illnesses such as meningitis, encephalitis, and sepsis (Schlech and Acheson, 2000). \textit{Listeriosis} normally causes serious complication among susceptible groups from neonates, infants, young children, pregnant women, elderly and immunocompromised patients (Buchanan et al., 2017; Jeyalatchumi et al., 2010). A large number of \textit{L. monocytogenes} ranging from 10 to 100 million of viable cells are required to cause illness in a healthy person. However, a relatively small number of the bacteria (0.1 – 10 million viable cells) is capable of causing illness in high risk groups (Bortolussi, 2008; Tauxe, 2001).

\textit{L. monocytogenes} infections are generally non-invasive that resulted in mild, febrile illness. The non-invasive infection generally required ingestion of a large number of \textit{L. monocytogenes} cells. Infected individuals will develop “flu-like” symptoms as well as fever and diarrhea (Aureli et al., 2000). However, it can cause invasive listeriosis which resulted in severe symptoms and fatality. The cases of invasive listeriosis were found to be more prevalent among high risk groups (Buchanan et al., 2017). Thus, the current risk assessment focused on invasive listeriosis among high risk groups. Dose-response relations model used to describe dose interaction, infectivity and the probability of infection from the exposure to the \textit{L. monocytogenes} was adapted from FAO/WHO (2004). In this model, each microbial cell is assumed to have an equal chance of causing infection. Walls (2006) highlighted the need to dose-response model which help to address the need of human being exposed to the hazard in order to calculate the probability.

Dose response model FAO/WHO (2004) used for estimating the probability of listeriosis in the study was as follow:

\[
P_{\text{infect}} = 1 - \exp^{-rN}
\]

where \( r \) is a variable that describes the dose/response relationship and \( N \) is the amount of microbes ingested.

This model makes assumption that every ingested pathogen will have equal probability (variable “\( r \)”) to cause infection within a particular population. The numbers of \textit{L. monocytogenes} cells used to calculate the probability of infection among high risk group was around 5 log CFU per serving based on the average dose capable of causing illness in high risk groups (Bortolussi, 2008; Tauxe, 2001). This model predicts the likelihood of getting foodborne illness based on the amount of pathogens ingested. Thus, the likelihood of listeriosis will remain the same regardless of the amount of cells ingested once the infective dose is achieved. The dose-response relationship values for different high risk group were summarized in Table 1.

**Table 1** Dose-response relationship for \textit{L. monocytogenes} for different high-risk groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose-response relationship</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td>8.5 x 10^{-6}</td>
<td>FAO/WHO (2004)</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>5.6 x 10^{-10}</td>
<td>Lindqvist and Westwoo (2000)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>2.6 x 10^{-11}</td>
<td>FDA/FSIS (2003)</td>
</tr>
<tr>
<td>Elderly, above 60 years of age</td>
<td>8.4 x 10^{-15}</td>
<td>FDA/FSIS (2003)</td>
</tr>
</tbody>
</table>

It was assumed that there were no seasonality and consumption pattern changes throughout the year (52 weeks). The probability of illness per year was calculated based on the formula below (Robertson et al., 2005):

\[
\text{Probability of illness per year} = 1 - (1 - P_{\text{infect}})\text{per individual})^{52}
\]

**Exposure assessment**

Exposure assessment requires data such as prevalence or concentration of microbes found in a specific food per serving (Walls, 2006). The exposure assessments for estimating the risk of \textit{L. monocytogenes} infection were based on the survivability of \textit{L. monocytogenes} on chicken liver, gizzard and heart performed in this study with reference to the previous study on the transmission of the bacteria from raw to cooked samples after contact with food contact surfaces (Goh et al., 2014).

Survivability of \textit{L. monocytogenes} on chicken liver, gizzard and heart

\textit{L. monocytogenes} growth on chicken liver, gizzard and heart were determined to simulate its survival patterns in cases of cross-contamination due to poor food handling. The level of \textit{L. monocytogenes} on chicken offal will affect the amount being transfer to the cooked chicken offal via food contact surfaces (Goh et al., 2014).

- Preparation of \textit{L. monocytogenes} inoculum

\textit{L. monocytogenes} ATCC 7944 was revived in 10 ml of Tryptic Soy Broth (TSB) (Merck, Germany) and incubated at 37°C for 24 h. The revived culture was streaked on PALCAM agar (Merck, Germany) and incubated at 37°C for 24 h to obtain a purified culture. Pure \textit{L. monocytogenes} culture was grown in TSB (Merck) in a condition described earlier before the culture was harvested for inoculum preparation. The inoculum of \textit{L. monocytogenes} was determined using spectrophotometer at 600 nm wavelength to give OD 0.99 which corresponds to 9.20 log CFU/ml.

- Survival determination of \textit{L. monocytogenes} on chicken offal

The survival determination study was carried out as described in our previous study with modification (Tang et al., 2014). Three grams of chicken liver, gizzard and heart were placed in universal bottles meant for different holding time (0, 1, 3, and 6 h). A total of 20 μl of inoculum was spiked onto the chicken offal and incubated at 28°C. \textit{L. monocytogenes} enumeration was performed using a spread plate method on PALCAM agar (Merck, Germany). The experiments were done in triplicates.
Data analysis

Data collected during the experiment was analyzed using SPSS 17.0 software. The data were analyzed using one-way ANOVA. The significance level was set at p < 0.05.

Transfer of *Listeria monocytogenes* to cooked sample

The transfer data of *Listeria monocytogenes* from raw to cooked sample was obtained from our previous study (Goh et al., 2014). Poor food handling which let to contaminated food has been recognized as one of the main factors which lead to the foodborne illness or outbreak (Soon, 2019). Goh et al. (2014) reported the transfer rate of *Listeria monocytogenes* to cooked sample ranging from 0 to 87% depending on the type of cutting boards and conditions of the cooked samples (hot or cooled). An average of 44% was used in this study to cover all the possible scenarios. The serving size of chicken offal consumption in Malaysia was based on data reported by Kuan et al. (2015) at 125 g per week.

Population of the high-risk groups

Based on the Malaysia population in year 2018 (Department of Statistics Malaysia, 2019), it was estimated 127,900 live births as of year 2018 and it was assumed that each live birth was from one pregnant woman (Department of Statistics Malaysia, 2019). Thus, it was assumed there were 127,900 pregnant women as of year 2018. Due to no data available for immunocompromised population in Malaysia, it is assumed 3.6% of the total population are immunocompromised which give around 1.2 million people fall within this group based on the estimation made by Laura (2008). Table 2 shows the population for each high-risk group in Malaysia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>Number of people</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>2018</td>
<td>32,600,000</td>
<td>Department of Statistics Malaysia (2019)</td>
</tr>
<tr>
<td>Immuno-compromised</td>
<td>NA</td>
<td>1,200,000</td>
<td>Laura (2008)</td>
</tr>
<tr>
<td>Pregnant women, elderly</td>
<td>2018</td>
<td>*127,900</td>
<td>Department of Statistics Malaysia (2019)</td>
</tr>
<tr>
<td>Elderly, above 60 years of age</td>
<td>2020</td>
<td>*2,347,200</td>
<td>Department of Statistics Malaysia (2006)</td>
</tr>
</tbody>
</table>

*It was assumed 3.6% of the total population are immunocompromised based on assumption made by Laura (2008).*

*It was projected 7.2% of the total population are elderly based on the projection made by Department of Statistics Malaysia (2006).*

**RESULTS AND DISCUSSION**

The growth of *Listeria monocytogenes* on chicken offal stored at room temperature (28°C) over 6 h period was summarized in Table 3. *Listeria monocytogenes* were found to grow significantly in chicken liver, gizzard and heart at 3 and 6 hours. There was no significant difference at 1 h. The survival pattern of *Listeria monocytogenes* was similar among chicken liver, gizzard and heart. The amount of *Listeria monocytogenes* at cooked chicken offal was calculated based on the average bacteria level at 6 h and transfer rate of 44% which corresponds to 3.02 log CFU/g or 1.04 x 10^3 CFU/g.

The calculated amount of *Listeria monocytogenes* (1.04 x 10^3 CFU/g) was used to calculate the probability of infection as shown in Table 4. Based on the dose-response model (FAO/WHO, 2004), it was found the immunocompromised group has the highest probability of infection per year at 3.78 x 10^-3 cases. This is followed by pregnant women 1.76 x 10^-3, elderly 5.68 x 10^-4 and general population 5.75 x 10^-5.

It is generally recognized that *Listeria monocytogenes* is capable of growing at chilled or refrigerator temperature (Omac, Moreira and Castell-Perez, 2018; Millilo et al., 2012). *Listeria monocytogenes* was found to grow on chicken liver, gizzard and heart after 3 h storage at room temperature (28°C) even though it is known to be psychrophilic bacteria. The finding is line with other studies that *Listeria monocytogenes* was capable of growing in various food stored at 25°C. *Listeria monocytogenes* was reported to grow 4 – 6 log CFU/g on celery and chicken salad stored at 25°C over 4 days period (Sahu et al., 2017). Studies have found significant *Listeria monocytogenes* contamination in chicken offal (Arumugaswamy et al., 1994; Kuan et al., 2013). Thus, the growth of *Listeria monocytogenes* on chicken offal at room temperature will increase the risk of cross contamination in the kitchen environment. Storage of chicken offal at temperature danger zone or under cooking may allow *Listeria monocytogenes* to survive and increase in number (Lin et al., 2004).

### Table 2 Population of high-risk groups in Malaysia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>Number of people</th>
<th>Reference</th>
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<td>2020</td>
<td>*2,347,200</td>
<td>Department of Statistics Malaysia (2006)</td>
</tr>
</tbody>
</table>

### Table 3 Growth of *Listeria monocytogenes* on chicken liver, gizzard and heart over 6 h incubation at room temperature.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6.08 ± 0.19</td>
<td>6.24 ± 0.06</td>
<td>6.61 ± 0.18</td>
<td>7.16 ± 0.08</td>
</tr>
<tr>
<td>Gizzard</td>
<td>5.97 ± 0.46</td>
<td>6.17 ± 0.37</td>
<td>6.44 ± 0.16</td>
<td>7.23 ± 0.48</td>
</tr>
<tr>
<td>Heart</td>
<td>5.21 ± 0.04</td>
<td>5.31 ± 0.08</td>
<td>5.56 ± 0.09</td>
<td>6.18 ± 0.07</td>
</tr>
</tbody>
</table>

Data represent mean ± standard deviation of three replications.

### Table 4 Risk assessment of *Listeria monocytogenes* in chicken offal consumption by high-risk groups in Malaysia.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>General population</th>
<th>Immuno-compromised</th>
<th>Pregnant women</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of <em>Listeria monocytogenes</em> (CFU/g)</td>
<td>1.04 x 10^3</td>
<td>1.04 x 10^3</td>
<td>1.04 x 10^3</td>
<td>1.04 x 10^3</td>
</tr>
<tr>
<td>Dose (log CFU/serving)</td>
<td>1.30 x 10^-5</td>
<td>1.30 x 10^-5</td>
<td>1.30 x 10^-5</td>
<td>1.30 x 10^-5</td>
</tr>
<tr>
<td>Probability of infection per serving</td>
<td>1.11 x 10^-10</td>
<td>7.28 x 10^-10</td>
<td>3.38 x 10^-6</td>
<td>1.09 x 10^-9</td>
</tr>
<tr>
<td>Probability of listeriosis cases per 100,000 population</td>
<td>2.87 x 10^-4</td>
<td>188.92</td>
<td>8.79 x 10^-3</td>
<td>2.84 x 10^-3</td>
</tr>
<tr>
<td>Probability of infection per year</td>
<td>5.75 x 10^-9</td>
<td>3.78 x 10^-3</td>
<td>1.76 x 10^-4</td>
<td>5.68 x 10^-8</td>
</tr>
</tbody>
</table>

### Table 5 Growth of *Listeria monocytogenes* on chicken offal stored at room temperature.

<table>
<thead>
<tr>
<th>Level of <em>Listeria monocytogenes</em> (log CFU/g)</th>
<th>0 h</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

Data represent mean ± standard deviation of three replications.

A, B, C Data in the same row with different letters is different significantly (p < 0.05).

A, B, C Data in the same column with different letters is different significantly (p < 0.05).

### Table 6 Risk assessment of *Listeria monocytogenes* in chicken offal consumption by high-risk groups in Malaysia.

Chicken offal is not generally consumed raw but chicken liver tends to be undercooked in order to retain its juiciness. Undercooking and contamination due to poor handling practices will contribute to the risk of listeriosis among high-risk groups. Poor food preparation practices have been identified as the contributing factor to these contamination (Soon, 2019).
Immunocompromised patients cover a variety of diseases that affect different components of the immune system. Immunocompromised patients can be categorized into congenital and acquired immunodeficiencies with the former being primary immunodeficiency diseases (PIDs) and the later cover range of disease from HIV/AIDS, cancer, hematologic and lymphatic malignancy, and others. Infection has been recognized as the main factor causing illness and death that required medical treatment (Mediani et al., 2014). From the risk estimates, it was found that this group is the most high risk group to get listeriosis. As such, this population group requires more vigilant in the selection of the uncontaminated food for consumption as low number of L. monocytogenes might cause serious illness. Brain infection cases due to L. monocytogenes has been reported in multiple sclerosis and stem cell transplant patients (Barocci et al., 2015; Tecelliglou et al., 2019).

Listeriosis is recognized as the most dangerous bacteria that cause death for fetuses and infants (Xu et al., 2017). A study reported women experiencing down-regulation of the immune system during pregnancy due to hormonal changes. Such changes increase the risk of foodborne illness among pregnant women (Smith, 1999). Pregnant women are generally 20 times greater risk of acquiring listeriosis than general population (Cook et al., 2018). Listeriosis can cause serious complication during pregnancy such as miscarriage, stillbirth, preterm labor, or death to the newborn (Xu et al., 2017). Listeriosis during pregnancy frequently occurs during the third trimester of pregnancy (Cook et al., 2018). This study found pregnant women were of higher risk of acquiring listeriosis than the elderly group was in agreement with a study done by Silk et al. (2012).

This risk assessment study proved high risk group should pay extra attention to this foodborne pathogen which can cause serious illness or even death. The incidence of listeriosis might be underreported due to mild clinical symptoms such as flulike fever and diarrhea. Microbiological examination was rarely performed for mild clinical symptoms and early pregnancy loses are generally not being investigated microbiologically (McLauchlin et al., 2004).

CONCLUSION

L. monocytogenes ability to grow in chicken offal stored at room temperature posed a significant risk of foodborne illness from inadequate cooking or contamination of cooked food. An immunocompromised group has the highest risk of listeriosis followed by pregnant women, elderly and general population. Risk assessment study based on the data from multiple sources from farm to table is important to provide information on the probability of illness resulted from contaminated food consumption by the high risk groups.

ACKNOWLEDGEMENT

The authors would like to thank the fund provider International Foundation of Sciences, Sweden (E/5237-2F). Special thanks to Universiti Putra Malaysia and Universiti Sultan Zainal Abidin, Malaysia for providing the facilities.

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